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(54) Title: GRAVES' OPHTHALMOPATHY ASSOCIATED ANTIBODIES, GRAVES' OPHTHALMOPATHY ORBITAL ANTIGEN, AND METHODS OF USE THEREOF

(57) Abstract

Graves' ophthalmopathy associated immunoglobulin and Graves' ophthalmopathy orbital antigen; immunoglobulin genes which are associated with autoimmunity in humans; products and processes involved in the cloning, preparation, and expression of genes for the immunoglobulin and antigen; antibodies directed against the ophthalmopathy associated immunoglobulin (anti-antibody and anti-idiotypic antibody) and antigen; methods of detecting the presence of the ophtalmopathy associated immunoglobulin and/or antigen(s); and pharmaceutical compositions containing anti-idiotypic antibodies, are disclosed. The present invention is also directed to isolated genomic DNA, cDNA, antisense RNA, and RNA containing the immunoglobulin or antigen(s) sequence.

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GRAVES' OPHTHALMOPATHY ASSOCIATED ANTIBODIES, GRAVES' OPHTHALMOPATHY ORBITAL ANTIGEN, AND METHODS OF USE THEREOF

The present invention was made utilizing funds of the United States Government. The United States Government may have certain rights in the invention.

Field of the Invention:

The present invention relates generally to the fields of immunology and recombinant genetics. More particularly, the present invention relates to Graves' ophthalmopathy associated antibody and Graves' ophthalmopathy orbital antigen; immunoglobulin genes which are associated with autoimmunity in humans; products and processes involved in the cloning, preparation, and expression of genes for the antibody and the antigen; and to diagnostic and therapeutic uses thereof.

BACKGROUND OF THE INVENTION

Graves' ophthalmopathy is a common, disfiguring autoimmune disorder of unknown etiology. Despite extensive investigations over many years, there is still no consensus on the target cell(s) or autoantigen(s) involved (Wall, J.R., *et al., Clin. Immunol. Immunopathol. 68*:18. (1993), Weetman, A.P., *Autoimmunity 12*:215- 222. (1992) and Kendler, D.L., *et al., Clin. Endocrinol. 35*:539- 547. (1991)).

Autoantibodies to orbital specific antigens in Graves' ophthalmopathy have long been sought but the evidence for such autoantibodies is controversial (reviewed in Weetman, A.P., *Thyroid associated ophthalmopathy, Autoimmunity 12*:215-222 (1992)). One of the major difficulties in screening sera for antibody binding to relatively crude antigen extracts or cultured cells is distinguishing a specific signal from the background. Accordingly, it is desirable to produce monoclonal antibodies, derived from plasma cells infiltrating Graves' orbital tissue (Weetman, A.P., *Thyroid associated ophthalmopathy, Autoimmunity 12*:215-222 (1992) and Campbell, R.J., *Immunology of Graves' ophthalmopathy retrobulbar histology and histochemistry, Acta Endocrinol., 121 (Suppl. 2)*:9-16 (1989)), to overcome this problem.

Because of the small number of tissue-infiltrating plasma cells and the limited amount of orbital tissue available, development of IgG-secreting B cell lines by the conventional approaches of cell fusion and/or EB virus transformation is unlikely to succeed. Recently, a novel approach for generating monoclonal antibodies has been described, namely the molecular cloning of immunoglobulin genes by the polymerase chain reaction (PCR) and expression of these genes in bacteria (Huse, W.D., Sastry, L., Iverson, S.A., Kang, A.S., Alting-Mees, M., Burton, D.R., *et al., Generation of a large combinatorial library of the immunoglobulin repertoire in phage lambda, Science 246*:1275-1281 (1989).

Thus, there is a present need to investigate whether orbital antibodies can be cloned using PCR to obtain heavy (H) and light (L) chain immunoglobulin products from Graves' orbital tissue cDNA, as well as to identify and characterize such immunoglobulin and their associated antigens.

SUMMARY OF THE INVENTION

The present invention relates to Graves' ophthalmopathy associated immunoglobulin proteins and orbital tissue antigen(s) and processes involved in the cloning, preparation and expression of genes for these immunoglobulins and antigen(s); antibodies (anti-antibody, anti-idiotypic antibody) with specificity to these immunoglobulins; antibodies with specificity to these antigen(s); and nucleotide probes corresponding to these immunoglobulin proteins and antigen(s). The present antibodies and probes are useful for detecting the presence of orbital tissue antigen in an orbital tissue sample. The present antigen polypeptide is useful for detecting Graves' ophthalmopathy antibodies in an orbital tissue sample as well as for producing antibodies thereto.

The present invention relates to the identification, characterization and sequencing of cDNA's and genomic fragments which encode Graves' ophthalmopathy immunoglobulin proteins and orbital tissue antigen(s), which are present in orbital tissue infiltrating B cells and orbital tissue antigens which are present in other orbital tissue cells, in patients with Graves' ophthalmopathy.

According to the invention, there are provided genetic sequences encoding the present immunoglobulin proteins. These proteins can be used to identify the present antigen. Once identified these antigen(s) can be isolated and the genetic sequences of the antigen(s) can be readily determined by one of ordinary skill in the art using known techniques. These antigen peptides and fragments thereof can then be used to detect the present immunoglobulin and to produce antibodies thereto. The invention also provides for expression vectors containing such genetic sequences, hosts transferred with such expression vectors, and methods for

producing the genetically engineered or recombinant immunoglobulin proteins and antigen polypeptid s.

The invention also provides antibodies which specifically recognize present immunoglobulin proteins (anti-antibody, anti-idiotypic antibody) and the present antigen(s).

The present immunoglobulin or antigen cDNA and recombinant protein are useful for making antibodies which specifically recognize the present immunoglobulin protein and antigen, respectively.

The present invention also relates to pharmaceutical compositions containing the present anti-idiotypic antibody raised against the present immunoglobulin protein(s).

The present invention further relates to methods of treating Graves' ophthalmopathy, whereby a therapeutically effective amount of a pharmaceutical composition containing the present anti-idiotypic antibody raised against the present Graves' ophthalmopathy associated immunoglobulin protein(s), is administered to a patient having Graves' ophthalmopathy.

The present invention also relates to immunoglobulin fragments of the present ophthalmopathy associated immunoglobulin whose genes are generally associated with autoimmunity, immunoglobulin fragments whose genes are generally associated with autoimmunity, and genes whose sequences are substantially homologous to such autoimmunity associated gene sequences; products and processes involved in the cloning, preparation and expression of such genes, and to diagnostic and therapeutic uses of such gene products. Such autoimmune associated genes include the germline genes KL012, DP10, hv1263, S4343 and DP54, and

genes whos sequences are substantially homologous thereto. Such autoimmune associated immunoglobulin fragments of the present ophthalmopathy associated immunoglobulin include OF7K.3, OF7K.16, OF7K.11, OF7K.9, OF7K.19, OF7K.17, OF7K.7, OF7H1.2 and OF7H1.19 having the sequences as shown in Figures 3-6.

An immunoglobulin heavy (H) chain and an immunoglobulin kappa light (L) chain cDNA library from the orbital tissue of a patient with active Graves' ophthalmopathy were constructed. Analysis of 15 H (IgG1) and 15 L (kappa) chains revealed a restricted spectrum of variable (V) region genes. 14/15 V kappa genes were - 94% homologous to the closest known germline gene, KL012. 13/15 H chain genes were 91% and 90% homologous to the closest germline genes, DP10 and hv1263, respectively. These germline genes also code for other autoantibodies to striated muscle (KL012) and thyroid peroxidase (KL012 and hv1263). The present inventors have discovered that particular germline genes and genes substantially homologous thereto, are associated with autoimmunity in humans.

DESCRIPTION OF THE FIGURES

Figure 1. Fig. 1 illustrates L chain gene PCR products from Graves' thyroid (T), blood (B), orbital fat tissues from 7 patients (F1-F7) and orbital muscle from patient 7 (M7). "-" Control lane lacking template. L-DNA ladder. Fig. 1(A) illustrates lambda L chain products obtained using V1 + C1 oligonucleotide primers. Fig. 1(B) illustrates kappa L chain products obtained using Vk + Ck primers. Fig. 1(C) illustrates beta-actin products.

Figure 2. Fig. 2 illustrates IgG1 H chain PCR products from blood (B), orbital fat (patients 1-7, F1-7) and orbital muscle from patient 7 (M7). "-" Control lane lacking template. L-DNA ladder. Fig. 2(A) illustrates IgG1 H chain products obtained for cDNA from blood (B), orbital F1-6, M7 and F7 using VH1a/3a and Cg1 oligonucleotide primers. Fig. 2(B) illustrates IgG1 H chain products obtained for cDNA from F1 and F7, M7 and Graves' thyroid (T) using Vc/d + Cg1 and VH6 + Cg1 primers.

Figure 3. Fig. 3 illustrates the nucleotide sequence (Fig. 3(A)) [SEQ ID NOS: 1 - 8] and the derived amino acid sequence (Fig. 3(B)) [SEQ ID NOS: 9 - 16] of the VK genes in clones OF7K.3, 9, 11, 16, 17 and 19. The closest putative germline gene, KL012 (Pargent, W., *et al., Eur. J. Immunol., 21*:1821- 1827 (1191)) is shown at the top. S43434 is a VK 1 gene used in a myasthenia gravis striational muscle autoantibody of thymic B cell origin (Victor, K.D., *et al., Eur. J. Immunol. 22*:2231-2236 (1992)). *Identical to OF7K.5, 10, 12, 15, 18, 20, 23 and 25.

Figure 4. Fig. 4 illustrates the nucleotide sequence (Fig. 4(A)) [SEQ ID NOS: 17 - 18] and the derived amino acid sequence (Fig. 4(B)) [SEQ ID NOS: 19 - 20] of the VK gene in clone OF7K.7. The closest putative germline gene, VK005 (Straubinger, B., *et al., J. Mol. Biol., 199*:23-24 (1988)) is shown at the top.

Figure 5. Fig. 5 illustrates the nucleotide sequence (Fig. 5(A)) [SEQ ID NOS: 21 - 23] and the derived amino acid sequence (Fig. 5(B)) [SEQ ID NOS: 24 - 26] of the VH gene in clone OF7H1.2. The closest putative germline gene, DP10 (Tomlinson, I.M., *et al., J. Mol. Biol., 227*:776-798 (1992)) and HV1263 (Chen, P.P., *et al., Arth. Rheum., 32*:72-76 (1989)) are shown at the top. Fig. 5(C) [SEQ ID NOS: 27 - 29] illustrates the nucleotide and the derived amino acid

sequences of the D region of OF7H1.2 group of H chain genes. The DHQ52 germline D region gene (Schroeder, H.W. Jr., *et al., Science, 238*:791-793 (1987)) is shown on top. *Identical to OF7H1.4, 5, 6, 7, 8, 10, 12, 15, 16, 18, 20 and 24.

Figure 6. Fig. 6 illustrates the nucleotide sequence (Fig. 6(A)) [SEQ ID NOS: 30 - 31] and the derived amino acid sequence (Fig. 6(B)) [SEQ ID NOS: 32 - 33] of the VH gene in clone OF7H1.19. The closest putative germline gene, DP54 (Tomlinson, I.M., *et al., J. Mol. Biol., 227*:776-798 (1992)) is shown at the top. Fig. 6(C) [SEQ ID NOS: 34 - 35] illustrates the nucleotide and the derived amino acid sequences of the D region of this group of H chain genes. *Identical to OF7H1.25.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

To aid in the understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

Gene. By the term "gene" is intended a DNA sequence which encodes through its template or messenger RNA a sequence of amino acids characteristic of a specific peptide. Further, the term includes intervening, non-coding regions, as well as regulatory regions, and can include 5' and 3' ends.

Gene sequence. The terminology "gene sequence" is intended to refer generally to a DNA molecule. As used herein, the terminology is meant to include both a DNA molecule which contains a non-transcribed or non-translated

sequence. The term is further intended to include any combination of gene(s), gene fragment(s), non-transcribed sequence(s), or non-translated sequence(s) which are present on the same DNA molecule.

The present sequences may be derived form a variety of sources including DNA, cDNA, synthetic DNA, RNA, or combinations thereof. Such gene sequences may comprise genomic DNA which may or may not include naturally occurring introns. Moreover, such genomic DNA may be obtained in association with promoter regions or poly A sequences. The gene sequences, genomic DNA or cDNA may be obtained in any of several ways. Genomic DNA can be extracted and purified form suitable cells (i.e., orbital tissue infiltrating B cells, orbital tissue cells) by means well known in the art. Alternatively, mRNA can be isolated from a cell and used to produce cDNA by reverse transcription or other means.

cDNA. The term "cDNA" is meant complementary or copy DNA produced from an RNA template by the action of RNA-dependent DNA polymerase (reverse transcriptase). Thus a "cDNA clone" means a duplex DAN sequence complementary to an RNA molecule of interest, carried in a cloning vector. This term includes genes from which the intervening sequences have been removed.

Recombinant DNA. By the terminology "recombinant DNA" is meant a molecule that has been recombined by in vitro splicing cDNA or a genomic DNA sequence.

Cloning. By the term "cloning" is meant the use of *in vitro* recombination techniques to insert a particular gene or other DNA sequence into a vector molecule. In order to successfully clone a desired gene, it is necessary to employ methods for generating DNA fragments, for joining the fragments to vector molecules, for introducing the composite

DNA molecule into a host cell in which it can replicate, and for selecting the clone having the target gene from amongst the recipient host cells.

cDNA Library. By "cDNA library" is meant a collection of recombinant DNA molecules containing cDNA inserts which together comprise the entire genome of an organism. Such a cDNA library may be prepared by methods known to those of skill, and described, for example, in Maniatis, *et al.*, *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 2d. ed. (1989). Generally, RNA is first isolated from the cells of an organism from whose genome it is desired to clone a particular gene. Preferred for the purposes of the present invention are mammalian, and particularly human, cell lines. A presently preferred vector for this purpose is the LAMBDA-ZAP vector.

Cloning Vehicle. A plasmid or phage DNA or other DNA sequence which is able to replicate in a host cell. The cloning vehicle is characterized by one or more endonuclease recognition sites at which such DNA sequences may be cut in a determinable fashion without loss of an essential biological function of the DNA, which may contain a marker suitable for use in the identification of transformed cells. Markers include for example, tetracycline resistance or ampicillin resistance. The word vector can be used to connote a cloning vehicle.

Expression Control Sequence. An "expression control sequence" is a sequence of nucleotides that controls or regulates expression of structural genes when operably linked to those genes. They include the lac systems, the trp system major operator and promoter regions of the phage lambda, the control region of fd coat protein and other

sequences known to control the expression of genes in prokaryotic or eukaryotic cells.

Expression vehicle. An "expression vehicle" is a vehicle or vector similar to a cloning vehicle but which is capable of expressing a gene which has been cloned into it, after transformation into a host. The cloned gene is usually placed under the control of (i.e., operably linked to) certain control sequences such as promoter sequences. Expression control sequences will by depending on whether the vector is designed to express the operably linked gene in a prokaryotic or eukaryotic host and may additionally contain transcriptional elements such as enhancer elements, termination sequences, tissue-specificity elements, and/or translational initiation and termination sites.

Operator. A DNA sequence capable of interacting with the specific repressor, thereby controlling the transcription of adjacent gene(s).

Promoter. The term "promoter" is intended to refer to a DNA sequence which can be recognized by an RNA polymerase. The presence of such a sequence permits the RNA polymerase to bind and initiate transcription of operably linked gene sequences.

Promoter region. The terminology "promoter region" is intended to broadly include both the promoter sequences as well as all gene sequences which may be necessary for the initiation of transcription. The presence of a promoter region is, therefrom sufficient to cause the expression of an operably linked gene sequence.

Operably Linked. As used herein, the terminology "operably linked" means that the promoter controls the initiation of expression of the gene. A promoter is

operably linked to a sequence of proximal DNA if upon introduction into a host cell the promoter determines the transcription of the proximal DNA sequence or sequences into one or more species of RNA. A promoter is operably linked to a DNA sequence if the promoter is capable of initiating transcription of that DNA sequence.

Prokaryote. The term " prokaryote" is meant to include all organisms without a true nucleus, including bacteria.

Eukaryote. The term "eukaryote" is meant to include organisms and cells which have a true nucleus, including mammalian cells.

Host. The term "host" is meant to include not only prokaryotes, but also such eukaryotes as yeast and filamentous fungi, as well as plant and animal cells. The term includes an organism or cell that is the recipient of a replicable expression vehicle.

Substantially homologous. The terminology "substantially homologous" as used herein refers to the ability of a first DNA sequence encoding Graves' ophthalmopathy associated antibodies or Graves ophthalmopathy orbital antigen, to hybridize to a second DNA sequence encoding the foregoing, respectively, under stringent conditions, for example, at about 0.1x sodium citrate sodium chloride buffer (SSC) at a temperature of about 65°C.

Substantially pure. The terminology "substantially pure" means that the protein/molecule of interest is essentially free from any other detectable biological constituents.

Functional Derivative. A "functional derivative" of the present antigen or antibody, is a protein which possesses a biological activity (either functional or structural) or immunological characteristics that are substantially similar to a biological activity or immunological characteristics of non- recombinant antigen or antibody. Such a functional derivative may or may not contain post-translational modifications such as covalently linked carbohydrate, depending on the necessity of such modifications for the performance of a specific function. The term "functional derivative is intended to include the "fragments," "variants," "analogues," "homologues," or "chemical derivatives" of a molecule.

Fragment. A "fragment" of a molecule such a the present antibody or antigen, is meant to refer to any variant of the molecule.

Variant. A "variant" of a molecule is meant to refer to a molecule substantially similar in structure and biological activity or immunological characteristics to either the entire molecule, or to a fragment thereof. Thus, provided that two molecules possess a similar activity, they are considered variants as that therm is used herein even if the composition or secondary, tertiary, or quaternary structure of one of the molecules is not identical to that found in the other, or if the sequence of amino acid residues is not identical.

Analog. An "analog" of a molecule is meant to refer to a molecule substantially similar in function to either the entire molecule or to a fragment thereof. As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties may improve the molecule' solubility, absorption, biological half life,

etc. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Moieties capable of mediating such effects are described in Remington's Pharmaceutical Sciences (1980). Procedures for coupling such moieties to a molecule are well known in the art.

Anti-Antibody. By the terminology "anti-antibody" is intended an antibody directed against antigenic determinants on other antibody (immunoglobulin) molecules.

Anti-Idiotypic Antibody. By the terminology "anti-idiotypic antibody" is intended an antibody directed against an idiotypic determinant of another antibody.

Idiotope. By the term "idiotope" is intended an idiotypic determinant, i.e., an antigenic determinant on a variable domain of an immunoglobulin molecule.

Idiotype. By the term "idiotype" is intended a set of one or more idiotopes that distinguish a clone of immunoglobulin producing cells from other clones. Idiotypes occur in the variable domains of immunoglobulin molecules and may be within, near to, or outside of the antigen binding site; antibodies to idiotypes located within or near the antigen biding site will prevent the immunoglobulin from combining with the antigen.

Idiotype-anti-idiotype Network. By the terminology "idiotype-anti-idiotype network" is intended a B-cell regulatory mechanism. Activation of a B cell results in a clone of plasma cells producing immunoglobulin of a single idiotype, which because it was previously present in very small quantities, can be recognized as "nonself" and results in the production of anti-idiotypic antibodies directed

against its idiotypic determinants. There can also be anti-anti-idiotypic antibodies directed against the second antibodies, antibodies directed against them, and so forth. These antibodies react with antigen receptors on B cells and T helper and suppressor cells, as well as with circulating antibodies, to enhance or suppress production of the initial antibody by various mechanisms.

Orbital Tissue Sample. Suitable orbital tissue samples include orbital fat, connective or muscle tissue samples.

II. Genetic Engineering of Graves' Ophthalmopathy Associated Immunoglobulin Proteins, Autoimmune Associated Immunoglobulin Fragments and Orbital Antigens

This invention comprises amino acid sequences of Graves' ophthalmopathy associated immunoglobulin proteins, orbital antigen, genetic sequences coding for such mRNA, expression vehicles containing the genetic sequences, hosts transformed therewith and recombinant immunoglobulin proteins or antigen(s) and antisense RNA produced by such transformed host expression. The invention further comprises antibodies directed against such immunoglobulin proteins or antigen(s).

The process for genetically engineering such immunoglobulin or antigen(s) sequences, according to the invention, is facilitated through the cloning of genetic sequences which are capable of encoding the peptide and through the expression of such genetic sequences. As used herein, the term "genetic sequences" is intended to refer to a nucleic acid molecule (preferably DNA). Genetic sequences which are capable of encoding the immunoglobulin proteins or antigen(s) are derived from a variety of sources. These

sources includ genomic DNA, cDNA, synthetic DNA, and combinations thereof. The preferred source of the genomic DNA or mRNA is orbital tissue infiltrating B cells or orbital tissue cells. The mRNA may then be used to obtain cDNA by techniques known to those skilled in the art. Probes may be synthesized based on the amino acid sequence of the immunoglobulin proteins or antigen(s) by methods known in the art.

The Graves' ophthalmopathy associated immunoglobulin protein, orbital antigen, or immunoglobulin fragment genomic DNA of the invention may or may not include naturally occurring introns. Moreover, such genomic DNA may be obtained in association with the 5' promoter region of the immunoglobulin proteins or antigen(s) gene sequences and/or with the 3' transcriptional termination region. Further, such genomic DNA may be obtained in association with the genetic sequences which encode the 5' non-translated region of the immunoglobulin protein or antigen mRNA and/or with the genetic sequences which encode the 3' non-translated region. To the extent that a host cell can recognize the transcriptional and/or translational regulatory signals associated with the expression of the mRNA and protein, then the 5' and/or 3' non-transcribed regions of the native gene, and/or, the 5' and/or 3' non-translated regions of the mRNA, may be retained and employed for transcriptional and translational regulation. Graves' ophthalmopathy immunoglobulin proteins or antigen(s) genomic DNA can be extracted and purified from the orbital tissue including orbital tissue infiltrating B cells of humans having Graves' ophthalmopathy, or from any cell that expresses the immunoglobulin protein or antigen(s) by means well known in the art (for example, see *Guide to Molecular Cloning Techniques*, S.L. Berger, *et al.*, eds., Academic Press (1987)).

Alternatively, immunoglobulin protein or antigen mRNA can be isolated from any cell which produces or expresses the immunoglobulin protein or antigen, and used to produce cDNA by means well known in the art (for example, see *Guide to Molecular Cloning Techniques,* S.L. Berger, *et al.*, eds., Academic Press (1987)). Preferably, the mRNA preparation used will be enriched in mRNA coding for such immunoglobulin protein or antigen(s), either naturally, by isolation from cells which are producing large amounts of the protein, or *in vitro*, by techniques commonly used to enrich mRNA preparations of specific sequences, including for example sucrose gradient centrifugation, or PCR. cDNA can then be prepared for example, by reverse transcription. The cDNA can then be amplified by PCR using suitable primers (Chazenbalk, G.D., *et al.*, *J. Clin. Invest.*, *92:*62-74 (1993).

For cloning into a vector, such suitable DNA preparations (either human genomic DNA or cDNA) are randomly sheared or enzymatically cleaved, respectively, and ligated into appropriate vectors to form a recombinant gene (either genomic or cDNA) library. A DNA sequence encoding the immunoglobulin protein or antigen(s) or its functional derivatives may be inserted into a DNA vector in accordance with conventional techniques, including blunt-ending or staggered-ending termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations are disclosed by Sambrook, *et al.*, (*In: Molecular Cloning, A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 2d. ed. (1989)), and are well known in the art.

Libraries containing the immunoglobulin protein and/or antigen(s) clones may be screened and an immunoglobulin and/

or antigen(s) clone can be identified by any means which specifically selects for immunoglobulin protein or antigen(s) DNA such as, for example, (a) by hybridization with an appropriate nucleic acid probe(s) containing a sequence specific for the DNA of this protein, or (b) by hybridization-selected translational analysis in which native mRNA which hybridizes to the clone in question is translated *in vitro* and the translation products are further characterized, or, (c) if the cloned genetic sequences are themselves capable of expressing mRNA, by immunoprecipitation of a translated immunoglobulin or antigen product produced by the host containing the clone.

Oligonucleotide probes specific for the immunoglobulin proteins or antigen(s) which can be used to identify clones to this protein can be designed from knowledge of the amino acid sequence of the immunoglobulin proteins or antigen(s). The sequence of amino acid residues in a peptide is designated herein either through the use of their commonly employed three-letter designations or by their single-letter designations. A listing of these three-letter and one-letter designations may be found in textbooks such as *Biochemistry*, Lehninger, A., Worth Publishers, New York, NY (1970). When the amino acid sequence is listed horizontally, the amino terminus is intended to be on the left end whereas the carboxy terminus is intended to be at the right end. The residues of amino acids in a peptide may be separated by hyphens. Such hyphens are intended solely to facilitate the presentation of a sequence.

Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid (Watson, J.D., *In: Molecular Biology of the Gene*, 3rd Ed., W.A. Benjamin, Inc., Menlo Park, CA (1977), pp. 356-357). The peptide fragments are analyzed to identify sequences of amino acids which may be encoded by oligonucleotides having

the lowest degree of deg neracy. This is preferably accomplished by identifying sequences that contain amino acids which are encoded by only a single codon.

Although occasionally an amino acid sequence may be encoded by only a single oligonucleotide sequence, frequently the amino acid sequence may be encoded by any of a set of similar oligonucleotides. Importantly, whereas all of the members of this set contain oligonucleotide sequences which are capable of encoding the same peptide fragment and, thus, potentially contain the same oligonucleotide sequence as the gene which encodes the peptide fragment, only one member of the set contains the nucleotide sequence that is identical to the exon coding sequence of the gene. Because this member is present within the set, and is capable of hybridizing to DNA even in the presence of the other members of the set, it is possible to employ the unfractionated set of oligonucleotides in the same manner in which one would employ a single oligonucleotide to clone the gene that encodes the peptide.

Using the genetic code (Watson, J.D., *In: Molecular Biology of the Gene*, 3rd Ed., W.A. Benjamin, Inc., Menlo Park, CA (1977)), one or more different oligonucleotides can be identified from the amino acid sequence, each of which would be capable of encoding the present immunoglobulin proteins or antigen(s). The probability that a particular oligonucleotide will, in fact, constitute the actual immunoglobulin or antigen coding sequence can be estimated by considering abnormal base pairing relationships and the frequency with which a particular codon is actually used (to encode a particular amino acid) in eukaryotic cells. Such "codon usage rules" are disclosed by Lathe, *et al.*, *J. Molec. Biol. 183*:1-12 (1985). Using the "codon usage rules" of Lathe, a single oligonucleotide sequence, or a set of oligonucleotide sequences, that contain a theoretical "most

probable" nucleotide sequence capable of encoding the immunoglobulin proteins and antigen(s) sequences is identified.

The suitable oligonucleotide, or set of oligonucleotides, which is capable of encoding a fragment of the immunoglobulin protein or antigen(s) gene (or which is complementary to such an oligonucleotide, or set of oligonucleotides) may be synthesized by means well known in the art (see, for example, *Synthesis and Application of DNA and RNA*, S.A. Narang, ed., 1987, Academic Press, San Diego, CA) and employed as a probe to identify and isolate the cloned immunoglobulin proteins or antigen(s) gene by techniques known in the art. Techniques of nucleic acid hybridization and clone identification are disclosed by Maniatis, *et al.* (*In: Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (1982)); Berger, *et al.*, (*In: Guide to Molecular Cloning Techniques*, Academic Press (1988)); Sambrook, *et al.*, (*In: Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY, 2d ed. (1989); and by Hames, *et al.* (*In: Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985)), which references are herein incorporated by reference. Those members of the above-described gene library which are found to be capable of such hybridization are then analyzed to determine the extent and nature of the immunoglobulin proteins or antigen(s) encoding sequences which they contain.

To facilitate the detection of the desired immunoglobulin protein or antigen DNA encoding sequence, the above-described DNA probe is labeled with a detectable group. Such detectable group can be any material having a detectable physical or chemical property. Such materials have been well-developed in the field of nucleic acid

hybridization and in general most any label useful in such methods can be applied to the present invention. Particularly useful are radioactive labels, such as ^{32}P, ^{3}H, ^{14}C, ^{35}S, ^{125}I, or the like. Any radioactive label may be employed which provides for an adequate signal and has a sufficient half-life. The oligonucleotide may be radioactively labeled, for example, by "nick-translation" by well-known means, as described in, for example, Rigby, *et al., J. Mol. Biol. 113*:237 (1977) and by T4 DNA polymerase replacement synthesis as described in, for example, Deen, *et al., Anal. Biochem. 135*:456 (1983).

Alternatively, polynucleotides are also useful as nucleic acid hybridization probes when labeled with a non-radioactive marker such as biotin, an enzyme or a fluorescent group. See, for example, Leary, *et al., Proc. Natl. Acad. Sci, USA 80*:4045 (1983); Renz, *et al., Nucl. Acids Res. 12*:3435 (1984); and Renz, M., *EMBO J. 6*:817 (1983).

Thus, in summary, the actual identification of immunoglobulin protein or antigen sequences permits the identification of a theoretical "most probable" DNA sequence, or a set of such sequences, capable of encoding such a peptide. By constructing an oligonucleotide complementary to this theoretical sequence (or by constructing a set of oligonucleotides complementary to the set of "most probable" oligonucleotides), one obtains a DNA molecule (or set of DNA molecules), capable of functioning as a probe(s) for the identification and isolation of clones containing the immunoglobulin protein or antigen gene.

In an alternative way of cloning the immunoglobulin protein or antigen gene, a library is prepared using an expression vector, by cloning DNA or, more preferably cDNA prepared from a cell capable of expressing the

immunoglobulin protein or antigen, into an expression vector. The library is then scre ned for members which express the immunoglobulin protein or antigen, for example, by screening the library with antibodies to the immunoglobulin protein or antigen protein.

The above discussed methods are, therefore, capable of identifying genetic sequences which are capable of encoding immunoglobulin proteins or antigen(s) or fragments thereof. In order to further characterize such genetic sequences, and, in order to produce the recombinant protein, it is desirable to express the proteins which these sequences encode. Such expression identifies those clones which express proteins possessing characteristics of the immunoglobulin proteins or antigen(s). Such characteristics may include the ability to specifically bind antibody to the immunoglobulin proteins or antigen(s) and the ability to elicit the production of antibody which are capable of binding to the immunoglobulin proteins or antigen(s).

III. Expression of Graves' Ophthalmopathy Immunoglobulin, Autoimmune Associated Immunoglobulin Fragments, Orbital Antigen, and Functional Derivatives Thereof

To express the present immunoglobulin or antigen polypeptide, transcriptional and translational signals recognizable by an appropriate host are necessary. The cloned immunoglobulin or antigen encoding sequences, obtained through the methods described above, and preferably in a double-stranded form, may be operably linked to sequences controlling transcriptional expression in an expression vector, and introduced into a host cell, either prokaryote or eukaryote, to produce recombinant immunoglobulin or antigen or a functional derivative thereof. Depending upon which strand of the immunoglobulin

or antigen encoding sequence is operably linked to the sequences controlling transcriptional expression, it is also possible to express immunoglobulin or antigen antisense RNA or a functional derivative thereof.

Expression of the immunoglobulin or antigen in different hosts may result in different post-translational modifications which may alter the properties of the immunoglobulin or antigen. The present invention encompasses the expression of the immunoglobulin or antigen, or a functional derivatives thereof, in prokaryotic or eukaryotic cells, and particularly, eukaryotic expression is preferred.

Preferred prokaryotic hosts include bacteria such as *E. coli*, *Bacillus*, *Streptomyces*, *Pseudomonas*, *Salmonella*, *Serratia*, etc. The most preferred prokaryotic host is *E. coli*. Other enterobacteria such as *Salmonella typhimurium* or *Serratia marcescens*, and various *Pseudomonas* species may also be utilized. Under such conditions, the protein may not be glycosylated. The procaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

To express the Graves' ophthalmopathy immunoglobulin or antigen protein (or a functional derivative thereof) in a prokaryotic cell (such as, for example, *E. coli*, *B. subtilis*, *Pseudomonas*, *Streptomyces*, etc.), it is necessary to operably link the Graves' ophthalmopathy immunoglobulin or antigen encoding sequence to a functional prokaryotic promoter. Such promoters may be either constitutive or, more preferably, regulatable (i.e., inducible or derepressible). Examples of constitutive promoters include the *int* promoter of bacteriophage lambda, the *bla* promoter of the Beta-lactamase gene of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene of pBR325,

etc. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage lambda (P_L and P_R), the *trp*, *recA*, *lacZ*, *lacI*, and *gal* promoters of *E. coli*, the alpha-amylase (Ulmanen, I., *et al.*, *J. Bacteriol. 162*:176-182 (1985)) and the sigma-28-specific promoters of *B. subtilis* (Gilman, M.Z., *et al.*, *Gene 32*:11-20 (1984)), the promoters of the bacteriophages of *Bacillus* (Gryczan, T.J., *In: The Molecular Biology of the Bacilli*, Academic Press, Inc., NY (1982)), and *Streptomyces* promoters (Ward, J.M., *et al.*, *Mol. Gen. Genet. 203*:468-478 (1986)). Prokaryotic promoters are reviewed by Glick, B.R., (*J. Ind. Microbiol. 1*:277-282 (1987)); Cenatiempo, Y. (*Biochimie 68*:505-516 (1986)); and Gottesman, S. (*Ann. Rev. Genet. 18*:415-442 (1984)).

Proper expression in a prokaryotic cell also requires the presence of a ribosome binding site upstream of the gene-encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold, L., *et al.* (*Ann. Rev. Microbiol. 35*:365-404 (1981)).

Especially preferred eukaryotic hosts are mammalian cells either *in vivo*, in animals or in tissue culture. Mammalian cells provide post-translational modifications to recombinant immunoglobulin or antigen which include folding and/or glycosylation at sites similar or identical to that found for the native immunoglobulin or antigen.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains expression control sequences which contain transcriptional regulatory information and such sequences are "operably linked" to the nucleotide sequence which encodes the polypeptide.

An operable linkage is a linkage in which a sequence is connected to a regulatory sequence (or sequences) in such a way as to place expression of the sequence under the influence or control of the regulatory sequence. Two DNA sequences (such as an immunoglobulin or antigen encoding sequence and a promoter region sequence linked to the 5' end of the encoding sequence) are said to be operable linked if induction of promoter function results in the transcription of the immunoglobulin or antigen encoding sequence mRNA and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the expression regulatory sequences to direct the expression of the immunoglobulin or antigen mRNA, antisense RNA, or protein, or (3) interfere with the ability of the immunoglobulin or antigen template to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence.

The precise nature of the regulatory regions needed for gene expression may vary between species or cell types, but shall in general include, as necessary, 5' non-transcribing and 5' non-translating (non-coding) sequences involved with initiation of transcription and translation respectively, such as the TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing control sequences will include a region which contains a promoter for transcriptional control of the operably linked gene.

Expression of the immunoglobulin or antigen in eukaryotic hosts requires the use of regulatory regions functional in such hosts, and preferably eukaryotic regulatory systems. A wide variety of transcriptional and translational regulatory sequences can be employed, depending upon the nature of the eukaryotic host. The

transcriptional and translational regulatory signals can also be derived from the genomic sequences of viruses which infect eukaryotic cells, such as adenovirus, bovine papilloma virus, Simian virus, herpes virus, or the like. Preferably, these regulatory signals are associated with a particular gene which is capable of a high level of expression in the host cell.

In eukaryotes, where transcription is not linked to translation, such control regions may or may not provide an initiator methionine (AUG) codon, depending on whether the cloned sequence contains such amethionine. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis in the host cell. Promoters from heterologous mammalian genes which encode mRNA product capable of translation are preferred, and especially, strong promoters such as the promoter for actin, collagen, myosin, etc., can be employed provided they also function as promoters in the host cell. Preferred eukaryotic promoters include the promoter of the mouse metallothionein I gene (Hamer, *et al., J. Mol. Appl. Gen.* *1*:273-288 (1982)); the TK promoter of Herpes virus (McKnight, S., *Cell 31*:355-365 (1982)); the SV40 early promoter (Benoist, *et al. Nature (London) 290*:304-310 (1981)); in yeast, the yeast *gal4* gene promoter (Johnston, *et al. Proc. Natl. Acad. Sci. USA 79*:6971-6975 (1982); Silver, *et al., Proc. Natl. Acad. Sci. USA 81*:5951-5955 (1984)) or a glycolytic gene promoter may be used.

As is widely known, translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and a DNA sequence which encodes the immunoglobulin or antigen, or a functional derivative thereof, does not contain any intervening codons which are capable of encoding a methionine. The presence of

such codons results either in the formation of a fusion protein (if the AUG codon is in the same reading frame as the immunoglobulin or antigen encoding DNA sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the immunoglobulin or antigen encoding sequence).

If desired, a fusion product of the immunoglobulin or antigen may be constructed. For example, the sequence coding for the immunoglobulin or antigen may be liked to a signal sequence which will allow secretion of the protein from or the compartmentalization of the protein in, a particular host. Such signal sequences may be designed with or without specific protease sites such that the signal peptide sequence is amenable to subsequent removal. Alternatively, the native signal sequence for this protein may be used.

Transcriptional initiation regulatory signals can be selected which allow for repression or activation, so that expression of the operably linked genes can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or are subject to chemical regulation, e.g., metabolite. Also of interest are constructs wherein the immunoglobulin or antigen mRNA and antisense RNA are provided in a transcribable form, but with different promoters or other transcriptional regulatory elements such that induction of immunoglobulin or antigen mRNA expression is accompanied by repression of antisense RNA expression, and/or repression of immunoglobulin or antigen mRNA expression is accompanied by induction of antisense RNA expression.

Translational signals are not necessary when it is desired to express immunoglobulin or antigen antisense RNA sequences.

If desired, the non-transcribed and/or non-translated regions 3' to the sequence coding for the immunoglobulin or antigen can be obtained by the above-described cloning methods. The 3'-non-transcribed region may be retained for its transcriptional termination regulatory sequence elements; the 3-non-translated region may be retained for its translation termination regulatory sequence elements, or for those elements which direct polyadenylation in eukaryotic cells. Where the native expression control sequence signals do not function satisfactorily in the host cell, then sequences functional in the host cell may be substituted.

The vectors of the invention may further comprise other operably linked regulatory elements such as enhancer sequences, or DNA elements which confer tissue or cell-type specific expression on an operably linked gene.

To transform a mammalian cell with the DNA constructs of the invention many vector systems are available, depending upon whether it is desired to insert the immunoglobulin or antigen DNA construct into the host cell chromosomal DNA, or to allow it to exist in an extrachromosomal form.

If the immunoglobulin or antigen DNA encoding sequence and an operably linked promoter is introduced into a recipient eukaryotic cell as a non-replicating DNA (or RNA) molecule, which may either be a linear molecule or a closed covalent circular molecule which is incapable of autonomous replication, the expression of the immunoglobulin or antigen

may occur through the transient expression of the introduced sequence.

Genetically stable transformants may be constructed with vector systems, or transformation systems, whereby immunoglobulin or antigen DNA is integrated into the host chromosome. Such integration may occur *de novo* within the cell or, in a most preferred embodiment, be assisted by transformation with a vector which functionally inserts itself into the host chromosome, for example, with retroviral vectors, transposons or other DNA elements which promote integration of DNA sequences in chromosomes. A vector is employed which is capable of integrating the desired gene sequences into a mammalian host cell chromosome.

Cells which have stably integrated the introduced DNA into their chromosomes are selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector in the chromosome, for example, the marker may provide biocide resistance, e.g., resistance to antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection.

In another embodiment, the introduced sequence is incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose, as outlined below.

Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector;

the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

Preferred eukaryotic plasmids include those derived from the bovine papilloma virus, vaccinia virus, SV40, and, in yeast, plasmids containing the 2-micron circle, etc., or their derivatives. Such plasmids are well known in the art (Botstein, *et al.*, *Miami Wntr. Symp. 19*:265-274 (1982); Broach, J.R., *In: The molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 445-470 (1981); Broach, J.R., *Cell 28*:203-204 (1982); Bollon, *et al.*, *J. Clin, Hematol. Oncol. 10*:39-48 (1980); Maniatis, T., *In: Cell Biology: A Comprehensive Treatise*, Vol. 3, "Gene Expression," Academic Pres, NY, pp. 563-608 (1980)), and are commercially available. For example, mammalian expression vector systems which utilize the MSV-LTR promoter to drive expression of the cloned gene, and in which it is possible to contransfect with a helper virus to amplify plasmid copy number, and integrate the plasmid into the chromosomes of host cells, have been described (Perkins, *et al.*, *Mol. Cell Biol. 3*:1123 (1983); Clontech, Palo Alto, California).

Once the vector or DNA sequence containing the construct(s) is prepared for expression, the DNA construct(s) is introduced into an appropriate host cell by any of a variety of suitable means, including transfection. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene sequence(s) results in the production of the immunoglobulin or antigen, or in the production of a fragment of this protein. This expression can take place in a continuous manner in the transformed cells, or in a controlled manner,

for example, expression which follows induction of differentiation of the transformed cells (for example, by administration of bromodeoxyuracil to neuroblastoma cells or the like).

The expressed protein is isolated and purified in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis, or the like.

The immunoglobulin or antigen DNA encoding sequences, obtained through the methods above, will provide sequences which, by definition, encode the immunoglobulin or antigen, or functional derivatives thereof and which may then be used to obtain immunoglobulin or antigen antisense RNA genetic sequences as the antisense RNA sequence will be that sequence found on the opposite strand of the strand transcribing the peptide core's mRNA. The antisense DNA strand may also be operably linked to a promoter in an expression vector such that transformation with this vector results in a host capable of expression of an immunoglobulin or antigen antisense RNA in the transformed cell. Antisense RNA and its expression may be used to interact with an endogenous immunoglobulin or antigen DNA or RNA in a manner which inhibits or represses transcription or translation of the immunoglobulin or antigen genes in a highly specific manner. Use of antisense RNA probes to block gene expression is discussed in Lichtenstein, C., *Nature 333*:801-802 (1988).

IV. Construction and Identification of Antibodies to raves' Ophthalmopathy Associated Immunoglobulin Autoimmune Associated Immunoglobulin Fragments and Orbital Antigen

In the following description, reference will be made to various methodologies well-known to those skilled in the art of immunology. Standard reference works setting forth the general principles of immunology include the work of Catty, D., (*Antibodies, A Practical Approach,* Vol. 1, IRL Press, Washington, DC (1988)); Klein, J., (*Immunology: The Science of Cell-Noncell Discrimination,* John Wiley & Sons, New York (1982)); Kennett, *et al.*, (*Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses,* Plenum Press, New York (1980)); Campbell, A. ("Monoclonal Antibody Technology," *In: Laboratory Techniques in Biochemistry and Molecular Biology,* Vol. 13 (Burdon, R., *et al.*, eds.), Elsevier, Amsterdam (1984)); and Eisen, H.N. (*In: Microbiology,* 3rd ed. (Davis, B.D., *et al.*, Harper & Row, Philadelphia (1980)).

An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. The term "epitope" is meant to refer to that portion of a hapten which can be recognized and bound by an antibody. An antigen may have one, or more than one epitope. An "antigen" is capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

The term "antibody" (Ab) or "monoclonal antibody" (Mab) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab and $F(ab)_2$ fragments) which are capable of binding an antigen. Fab and $F(ab)_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl, *et al., J. Nucl. Med. 24*:16-325 (1983)).

The antibodies of the present invention have specificity to one or more epitopes present on the antigen peptide, or an idiotype on the present immunoglobulin. The antibodies of the invention can be polyclonal or monoclonal, provided that they are made with the present immunoglobulin or antigen polypeptide or fragment thereof an immunogen. Both of these types of antibodies as well as the present Graves' ophthalmopathy associated immunoglobulin itself, can be utilized in the multiple applications described herein below.

The present antibodies can be used to detect the presence of the present immunoglobulin or antigen in a orbital tissue sample. The present immunoglobulin can be detected by contacting the sample with an imaging-effective amount of the present detectably labeled appropriate antibody and detecting the label, thereby establishing the presence of the present immunoglobulin or antigen in the sample. Detection can be carried out by imaging *in vivo*. The present immunoglobulin or antigen can also be detected by known immunoassay techniques, including, for example, RIA, ELISA, etc., using the present appropriate antibodies.

The antibodies of the present invention are prepared by any of a variety of methods. For example, cells expressing the present immunoglobulin or antigen, or a fragment thereof, can be administered to an animal in order to induce

the production of sera containing polyclonal antibodies that are capable of binding the present immunoglobulin or antigen. For example, an immunoglobulin or antigen fragment is chemically synthesized and purified by HPLC to render it substantially free of contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of high specific activity.

Polyclonal antibodies can be generated in any suitable animal including, for example, mice, rabbits or goats. The present immunoglobulin or antigen peptide can be injected by itself or linked to appropriate immunoactivating carriers, such as Keyhole's limpet hemocyanin (KLH). See *Antibodies, A Practical Handbook*, Vols. I and II, D. Catty, ed., IRL Press, Washington, DC (1988).

Monoclonal antibodies can be prepared in various ways using techniques well understood by those having ordinary skill in the art. For example, monoclonal antibodies can be prepared using hybridoma technology (Kohler, *et al., Nature 256*:495 (1975); Kohler, *et al., Eur. J. Immunol. 6*:511 (1976); Kohler, *et al., Eur. J. Immunol. 6*:292 (1976); Hammerling, *et al., In: Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)); *Monoclonal Antibodies - Hybridomas: A New Dimension in Biological Analysis*, edited by Roger H. Kennett, *et al.*, published by Plenum Press (1980). In general, such procedures involve immunizing an animal with the present immunoglobulin or orbital antigen, of a fragment thereof. The splenocytes of such animals are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands, *et al., Gastroenterology 80*:225-232 (1981), which is herein incorporated by reference. The hybridoma cells

obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the present Graves' ophthalmopathy associated immunoglobulin or orbital antigen.

Through application of the above-described methods, additional cell lines capable of producing antibodies which recognize epitopes of the present immunoglobulin or antigen can be obtained.

For example, additional hybridomas which produce monoclonal antibodies which enable the detection of the present immunoglobulin or antigen can be easily produced and isolated with minimal screening. Hybridomas producing monoclonal antibodies specific for epitopes which are found on the present immunoglobulin or orbital antigen are most effectively produced by first immunizing an animal from which hybridomas can be produced such as, for example, a Balb/c mouse, with initial subcutaneous injections of Freund's adjuvant, followed by booster injections within a few days. The fusion can be carried out using any of the techniques commonly known to those of ordinary skill in the art. The screening of the hybridomas to determine which ones are producing monoclonal antibodies specific for the present peptide is straightforward and can be done either in a standard ELISA or RIA format. For example, in an RIA screening format the culture supernatant, or ascites fluid from a hybridoma producing monoclonal antibody is reacted with ^{125}I-peptide. The isolation of other hybridomas secreting mAbs of the same specificity as those described herein can be accomplished by the technique of anti-idiotypic screening. Potocmjak, *et al.*, *Science 215*:1637 (1982). Briefly, an anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody can be prepared by immunizing an animal of the

same species and genetic type (e.g., mouse strain) as the source of the mAb with the mAb raised against the present antigen or with the present ophthalmopathy associated immunoglobulin to which an anti-Id is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody).

By using an anti-Id antibody which is specific for idiotypic determinants on a given mAb, it is then possible to identify other B cell or hybridoma clones sharing that idiotype. Idiotypic identity between the antibody product of two clones makes it highly probable that the antibody products of the two clones recognize the same antigenic epitopes.

The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may be epitopically identical to the original mAb which induced the anti-Id.

Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

Accordingly, mAbs generated against the present antigen may be used to induce anti-Id antibodies in suitable animals, such as BALB/c mice. Spleen cells from such immunized mice are used to produce anti-Id hybridomas secreting anti-Id mAbs. Further, the anti-Id mAbs can be coupled to a carrier such as keyhole limpet hemocyanin (KLH) and used to immunize additional BALB/c mice. Sera from these mice will contain anti-anti-Id antibodies that have the binding properties of the original mAb specific for the

antigen epitope. The anti-Id mAbs thus have their own idiotypic epitopes, or "idiotopes" structurally similar to the epitope being evaluated.

For replication, the hybridoma cells of this invention may be cultivated *in vitro* or *in vivo*. Production of high titers of mAbs *in vivo* production makes this the presently preferred method of production. Briefly, cells from the individual hybridomas are injected intraperitoneally into pristane-primed BALB/c mice to produce ascites fluid containing high concentrations of the desired mAbs. MAbs of isotype IgM or IgG may be purified from such ascites fluids, or from culture supernatants, using column chromatography methods well known to those of skill in the art.

Of special interest to the present invention are antibodies which are produced in humans, or are "humanized" (i.e., non-immunogenic in a human) by recombinant or other technology such that they will not be antigenic in humans, or will be maintained in the circulating serum of a recipient for a longer period of time.

Humanized antibodies may be produced, for example by replacing an immunogenic portion of an antibody with a corresponding, but non-immunogenic portion (i.e., chimeric antibodies) (Robinson, *et al.*, International Patent Publication PCT/US86/02269; Akira, *et al.*, European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison, *et al.*, European Patent Application 173,494; Neuberger, *et al.*, PCT Application WO 86/01533, Cabilly, *et al.*, European Patent Application 125,023; Better, *et al.*, *Science 240*:1041-1043 (1988); Liu, *et al.*, *Proc. Natl. Acad. Sci. USA 84*:3439-3443 (1987); Liu, *et al.*, *J. Immunol. 139*:3521-3526 (1987); Sun, *et al.*, *Proc. Natl. Acad. Sci. USA 84*:214-218 (1987); Nishimura, *et al.*,

Canc. Res. 47:999-1005 (1987); Wood, *et al.*, *Nature* 314:446-449 (1985)); Shaw, *et al.*, *J. Natl. Cancer Inst.* 80:1553-1559 (1988). General reviews of "humanized" chimeric antibodies are provided by Morrison, S.L. (*Science*, 229:1202-1207 (1985)) and by Oi, *et al.*, *BioTechniques* 4:214 (1986)).

Suitable "humanized" antibodies can be alternatively produced as described by Jones, *et al.*, *Nature* 321:552-525 (1986); Verhoeyan, *et al.*, *Science* 234:1534 (1988), and Beidler, *et al.*, *J. Immunol.* 141:4053-4060 (1988).

The present Graves' ophthalmopathy associated immunoglobulin, fragments thereof or autoimmune associated fragments thereof, or autoimmune associated immunoglobulin fragments, orbital antigen, or antibodies thereto can be utilized in immunoassays for the detection of the orbital antigen or ophthalmopathy associated immunoglobulin, respectively, in an orbital tissue sample. For example, the present immunoglobulin or fragment thereof, can be used to detect the present orbital antigen; the present orbital antigen, or fragment thereof, can be used to detect antibodies thereto in an orbital tissue sample; or antibodies against the present immunoglobulin can be used to detect the present immunoglobulin in an orbital tissue sample, etc. Autoimmune associated fragments of the present invention can be used to generally detect an antigen associated with an autoimmune condition such as thyroid peroxidase in Graves' disease. The immunoassays can be competitive or sandwich, or as is otherwise well known and they all depend on the formation of antibody-antigen immune complex. These assays are well known to those of skill in the art.

For purposes of the assays, the antibodies or antigen can be immobilized or labeled. There are many carriers to which the antibodies/antigen can be bound for immobilization and which can be used in the present invention. Well-known carriers include but are not limited to glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for purposes of the invention. Those skilled in the art will know many other suitable carriers for binding the antibodies or antigen peptide(s), or will be able to ascertain such, using routine experimentation.

Depending on the particular embodiment of the invention, one or more of the antibodies or antigen(s) peptide(s) will be coupled with a detectable label such as an enzyme, radioactive isotope, fluorescent compound, chemiluminescent compound, or bioluminescent compound.

Those of ordinary skill in the art will know of other suitable labels for binding to the antibodies or antigen(s) peptide(s) or will be able to ascertain such using routine experimentation. Furthermore, the binding of these labels to the antibodies or antigen(s) can be done using standard techniques
commonly known to those of ordinary skill in the art.

The antibodies or antigen peptide(s) can be bound to an enzyme. This enzyme, in turn, when later exposed to its substrate will react to the substrate in such a manner as to produce a chemical moiety which can be detected, as, for example, spectrophotometric or fluorometric means. Examples of enzymes that can be used to detectably label are amalate dehydrogenase, staphylococcal nuclease,

delta-5-steroidisomerase, yeastalcoholdehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholinesterase.

The presence of an antibody or antigen can also be detected by labeling the antibody or antigen peptide with a radioactive isotope. The presence of the radioactive isotope could then be determined by such means as the use of a gamma counter or a scintillation counter. Isotopes which are particularly useful are ^{3}H, ^{125}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{36}Cl, ^{57}Co, ^{59}Fe, ^{75}Se, and ^{152}Eu.

It is possible to detect the presence of the antibody or antigen by labeling the antibody or antigen peptide with a fluorescent compound. When the fluorescently labeled antibody or antigen peptide is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence of the dye. Among the most important fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, and fluorescamine.

Another way in which the antibody or antigen peptide can be detectably labeled is by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody or antigen peptide is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, aromatic-acridinium ester, imidaxole, acridinium salt, and oxalate ester. Other suitable chemiluminescent labels, are described in the following U.S. co-pending applications: U.S. Serial No. 07/

933,478, 07/859,955, 07/963,408, 07/943,185, 07/860,410, 07/859,676, 07/860,001, 07/859,956 and 07/859,994, all of which are hereby incorporated by reference in their entireties as though set forth in full.

Likewise, a bioluminescent compound may also be used to label the antibody or antigen peptide. Bioluminescence is a special type of chemiluminescence which is found in biological systems and in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent binding partner would be determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase, and aequorin.

The antibodies or antigen peptide(s) for use in the assay of the invention are ideally suited for the preparation of a kit. such a kit may comprise a carrier means being compartmentalized to receive in close confinement one or more container means such as vials, tubes, and the like, each of said container means comprising one of the separate elements to be used in the method.

For example, one of the container means may comprise a first antibody bound to an insoluble or partly soluble carrier. A second container may comprise soluble, detectalby-labeled second antibody, in lyophilized form or in solution. The carrier means may also contain a third container means comprising a detectalby-labeled third antibody in lyophilized form or in solution. Such a kit can be used for sandwich assays. See, e.g., David, *et al.*, USP 4,376,110 herein incorporated by reference.

In addition, the carrier means may also contain a plurality of containers each of which comprises different, predetermined amounts of the present antigen peptide. These

latter containers can then be used to prepare a standard curv into which can be interpolated the results obtained from the sample containing the unknown amount of the present orbital antigen.

Imaging can be carried out *in vitro* or *in vivo*. *In vitro* imaging can be done with the labels mentioned previously. *In vivo* imaging is done with diagnostically effective labeled antibodies or antigen(s). The term "diagnostically effective" means that the amount of detectably labeled antibody or antigen administered is sufficient to enable detection of the site of orbital antigen or ophthalmopathy asociated antibody presence when compared to a background signal.

Generally, the dosage of detectably labeled antibody or antigen(s) for diagnosis will vary depending on considerations such as age, condition, sex, and extent of disease in the patient, counterindications, if any, and other variables, to be adjusted by the individual physician. Dosage can very from 0.01 mg/kg to 2,000 mg/kg, preferably 0.1 mg/kg to 1,000 mg/kg.

The term "diagnostically labeled" means that the immunoglobulin or antigen has attached to it a diagnostically detectable label.

There are many different imaging labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include radioactive isotopes and paramagnetic isotopes.

For diagnostic *in vivo* imaging, the type of detection instrument available is a major factor in selecting a given radionuclide. The radionucleotide chosen must have a type

of decay which is detectable for a given type of instrument. In general, any conventional method for visualizing diagnostic imaging can be utilized in accordance with this invention.

Another important factor in selecting a radionuclide for *in vivo* diagnosis is that the half-life of a radionucleotide be long enough so that it is still detectable at the time of maximum uptake by the target, but short enough so that deleterious radiation upon the host is minimized. Ideally, a radionuclide used for *in vivo* imaging will lack a particulate emission, but produce a large number of photons in a 140-200 ke V range, which may be readily detected by conventional gamma cameras.

For *in vivo* diagnosis, radionucleotides may be bound to antibody or antigen either directly or indirectly by using an intermediary functional group. Intermediary functional groups which are often used to bind radioisotopes which exist as metallic ions to antibody or antigen are diethylenetriaminepentaacetic acid (DTPA) and ethlenediaminetetracetic acid (EDTA). Typical examples of metallic ions which can be bound to immunoglobulins are ^{99m}Tc, ^{123}I, ^{111}In, ^{131}I, ^{97}Ru, ^{67}Cu, ^{67}Ga, ^{72}As, ^{89}Zr, and $^{201}T1$.

The antibodies or antigen(s) used in the method of the invention can also be labeled with paramagnetic isotopes for purposes of *in vivo* diagnosis. Elements which are particularly useful (as in magnetic resonance imaging (MRI) techniques) in this manner include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, and ^{56}Fe.

Preparations of the imaging antibodies or antigen(s) for administration include sterile aqueous or non-aqueous

solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propyleneglycol, polyethyleneglycol, vegetable oil such as olive oil and injectable organic esters such as ethyloleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media, parenteral vehicles including sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. See, generally, *Remington's Pharmaceutical Science*, 16th ed. Mac Eds. 1980.

V. Pharmaceutical Compositions

Pharmaceutical compositions containing a therapeutically effective amount of the present anti-idiotypic antibody raised against the present Graves' ophthalmopathy associated antibody, are useful for treating patients with Graves' ophthalmopathy. Such compositions contain a therapeutically effective amount of the foregoing anti-idiotopic antibody, and may optionally contain one or more suitable pharmaceutically acceptable carriers and/or excipients, known to those of ordinary skill in the art. Administration, dosage and frequency, and length of treatment can be readily optimized for a particular patient by one of ordinary skill in the art. For example, the present pharmaceutical composition can be formulated as a sterile aqueous or non-aqueous suspensions or emulsions, as described above in Section IV, for example for solutions for intravenous administration.

Example 1

Amplification by Polymerase Chain Reaction Of Immunoglobulin Heavy and Light Chain Genes From Orbital Tissue of Patients With Graves' Ophthalmopathy

Materials and Methods

Orbital fat/connective tissue was obtained from 7 patients (F1-7) and orbital muscle from 1 patient (M7) undergoing orbital decompression for Graves' exphthalmos. mRNA was obtained from orbital tissues (~0.1g) using the QuickPrep mRNA Purification kit (Pharmacia, Piscataway, NJ) and cDNA was prepared by reverse transcription with oligo-dT (First Strand Synthesis kit: Stratacyte, La Jolla, CA). To control for the potential contribution of lymphocytes in the blood of orbital tissue, mRNA and cDNA were prepared by the same methods from an excess volume of blood (1 ml) of a normal donor. Thyroid cDNA from a Graves' patient (JA) (Chazenbalk, G.D., *et al., J. Clin. Invest., 92*:62-74 (1993)) was used as a positive control. The conditions and oligonucleotide primers for PCR amplification of H and L chain DNA were essentially as previously described in Chazenbalk, G.D., *et al., J. Clin. Invest., 92*:62-74 (1993) with two differences: (i) wax 'gems" (Perkin Elmer Cetus, Norwalk, Connecticut) were used to facilitate a "hot start"; (ii) Primers for amplification of lambda L chains were obtained from Stratacyte. Aliquots of tissue cDNA were assessed for beta-actin using oligonucleotide primers and PCR conditions described by Butch, A.W., *et al., J. Immunol. 150*:39- 47 (1993). PCR products (10%) were analyzed pm 1% agarose gels (3% NuSieve for actin) and visualized using ethidium bromide.

R sults and Discussion

Lambda L chain DNA was amplified from 6/7 orbital fat samples (F1, F3-F7) but not from F2 or from blood (Fig. 1(A)). In contrast, Kappa L chain DNA was only amplified using cDNA from orbital fat from two patients (F6 and F7), as well as muscle from one patient (M7) (Fig. 1(B)). Both L chains were amplified from the orbital muscle of one patient (M7). Beta-actin was amplified from all cDNA templates (Fig. 1(C)), indicating the integrity of the mRNA even in the samples not providing a lambda L chain signal (F2 and blood).

The preferential amplification of lambda DNA rather than kappa DNA in the same cDNA preparation from some orbital tissue cDNA is of interest. Individual plasma cells secrete either kappa or lambda L chains but not both. Preferential lambda amplification relates to more efficient priming for V lambda than for V kappa genes. However, if the bias is genuine, it resembles the lambda L chain bias demonstrated immunohistochemically in Graves' thyroid tissue (Jasani, B., *et al., J. Endocrinol. Invest. 9 (Suppl.3)*:101-101 (1986)).

Using an oligonucleotide primer (V1a/3a (Chazenbalk, G.D., *et al., J. Clin. Invest. 92*:62-74 (1993)), homologous with the 5' regions of the largest VH gene families VH1 and VH3, and a downstream primer (Cg1 (Chazenbalk, G.D., *et al., J. Clin. Invest. 92*:62-74 (1993)) for the constant region of IgG1, only DNA products with orbital muscle cDNA (M7) and orbital fat cDNA (F7) from patient 7 (Fig. 2(A)), were obtained. Detectable, but much lower, signals were observed with orbital fat cDNA from a few other patients.

Additional VH primers (Chazenbalk, G.D., *et al., J. Clin. Invest. 92*:62-74 (1993)) were used to investigate amplification of H chain DNA from three cDNA's M7, F7 and F1, because these were good templates for amplification of L chain DNA (Fig. 1). A representative experiment using the Vc/d and VH6 primers is shown in Fig. 2(B). IgG1 H chain DNA was amplified from M7 and F7 using all the VH primers except from M7 with the Vc/d primer. These data reflect the absence of certain VH genes in M7. No IgG1 H chain DNA was clearly amplified from F1 cDNA using any of the 5 VH region primers (Chazenbalk, G.D., *et al., J. Clin. Invest. 92*:62-74 (1993)) (Fig. 2(A) and (B)). The difficulty in amplifying H chain compared with L chain DNA from orbital tissue arises because mRNA isolation and reverse transcription using oligo-dt is less efficient for complete H chains which (unlike the 2-domain L chains) consist of 4 domains (VH and CH1, CH2, CH3). H and L chain DNA of the size observed can only be amplified form mRNA-derived cDNA. This experiment evidences that L and H chain DNA can be amplified by PCR from orbital tissue of some patients with Graves' ophthalmopathy.

Example 2

Molecular Cloning and Characterization of Genes for Antibodies Generated by Orbital Tissue Infiltrating B Cells in Graves' Ophthalmopathy

Introduction

In this example, libraries were constructed from the DNA amplified in example 1, in a bacteriophage vector and the nucleotide sequences of the V regions of 15 H and 15 L chains randomly selected from these libraries, were determined.

Materials and M thods

A. Construction of immunoglobulin H and L chain cDNA libraries

DNA amplified in the PCR reaction from cDNA generated by reverse transcription of mRNA isolated from orbital fat/ connective tissue of patient no. 7 (example 1), was used. This patient was a 62 year old male undergoing orbital decompression for malignant Graves' exophthalmos. The oligonucleotide primers used in the PCR are described in example 1. To cover all 6 VH gene families, 5 sets of forward primers were used, including those of Persson, M.A., *et al., Proc. Natl. Acad. Sci. USA, 88*:2432-2436 (1991), Marks, J.D., *et al., Eur. J. Immunol., 21*:985-991 (1991) and also the VHc/d primers of Stratacyte (La Jolla, CA). The reverse primer (Cg1) hybridizes with the CH1 domain-hinge junction of IgG1 H chains. The kappa L chain forward primer cross primes with the four VK gene families (Kabat, E.A., *et al., Sequences of proteinsof immunological interest*, U.S. Department of Health and Human Services, Ed. 5 (1991) and the reverse primer was to the carboxyl terminus of the C region. All primers contain restriction sites for cloning into the Imminozap bacteriophage vectors (Stratacyte).

H and L chain DNA was restricted with Xho I/Spe I and Sac I/Xba I, respectively, gel purified and then ligated into the Immunozap H and L lambda bacteriophage vectors as described in Portolano, S., *et al., Biochem. Biophys. Res. Comm. 179*:372-379 (1991) and Portolano, S., *et al., J. Clin. Invest. 90*:720-726 (1992). The unamplified H and L chain libraries each contained about 10^6 recombinants.

B. Nucleotide sequencing of random H and L chain genes

Randomly selected plaques were used to generate phagemid containing the respective cDNA inserts, as previously described in Portalano, supra. Plasmids were prepared from XL1-blue bacteria (Stratagene) infected with the phagemid. Nucleotide sequencing of plasmid cDNA inserts was performed by the dideoxynucleotide termination method described in Sanger, F., *et al., Proc Natl. Acad. Sci. USA* 74:5463-5467 (1977).

Results and Discussion

Immunoglobulin H and L chain genes are formed by the combination of a number of smaller genes separated by introns in the germline DNA (reviewed in Brodeur, P.H., *Genes encoding the immunoglobulin variable regions. In: Molecular Genetics of Immunoglobulin*, edited by Calabi, F. and Newberger, M.S.: Elsevier Science Publishers B.V., P. 81-84 (1987)). The antigen binding, or variable (V) region of the human H chain is formed by the combination of one of ~100 VH genes, 1 of ~20 diversity (D) genes and 1 of 6 joining (JH) genes. This VDJ unit is then joined to the constant region of the H chain. Similarly, the variable region of the human kappa L chain is formed form 1 of ~100 VK genes and 1 of 6 JK genes. The combination of multiple units itself generates diversity in the antigen-binding region. Further, diversity is introduced by antigen-driven somatic mutation in these germline genes. Therefore, as described below for orbital tissue immunoglobulin genes, each antibody can be characterized in terms of the germline (or putative germline) genes encoding the VH, D and JH regions of the H chain and the VJ and JK regions of the kappa L chain.

Analysis of random H and L kappa V gene sequence from the Graves' orbital tissue revealed them to be quite restricted. Two groups of L chains were identified among the V regions of the 15 kappa genes (Table I, Fig. 3). A dominant cluster of 14 VK genes contained sequences that were identical or highly related, differing by no more than 4 base pairs from one another. These genes were ~94% homologous to the closest known germline gene, L4.KL012, a VK1 (Pargent, W., *et al., Eur. J. Immunol. 21*:1821-1827 (1991). One of the orbital tissue KL012-like VK genes (OF7K.17) was >96% homologous to VK gene S43434 (Fig. 3) used by a striational muscle autoantibody derived from thymic B cells of a patient with myastheria gravis (Victor, K.D., *et al., Eur. J. Immunol. 22*:2231-2236 (1992). Graves' disease occurs in 3-5% of the patients with myasthenia gravis, while about 1% of patients with Graves' disease develop myasthenia gravis (Larsen, R.P., *et al., The Thyroid Gland.* In: Textbook of Endocrinology, edited by Wilson, J.D. and Foster, D. W. Philadelphia: W. B. Saunders Co., pp. 357-487 (1991). Although the patient had no symptoms of myasthenia gravis, thymic enlargement is frequently associated with Graves' disease (Michis, W., *et al., Lancet. i*:691-695 (1967).

The second VK gene group (Fig. 4) had only one representative (OF7K.7), which was 91% homologous to a VK2 germline gene, VK005 (Straubinger, B., *J. Mol. Biol. 199*:23-34 (1988). The two groups of VK genes were combined with different JK segments; JK2 for the KL 012 L chains and JK1 for the VK005 L chain (Table 1).

The H chain V regions could also be classified into two groups. One group, with closest homology (91%) to VH1 germline gene DP10 (Tomlinson, I.M., *J. Mol. Biol. 227*:776-798 (1992)), had thirteen identical representatives (Table 1, Figs. 5 (A) and (B)). Without determining the

relevant genomic nucleotide sequence in the patient, it is not certain that DP10 is the origin of this group of genes. The 14 orbital tissue VH genes are only slightly less homologous (90%) to another VH germline gene, hv1263 (Chen, P.P., *et al.*, *Arth. Rheum. 32*:72-76 (1989)), the putative origin of a number of thyroid peroxidase (TPO) autoantibody VH genes (Chazenbalk, G.D., *et al.*, *J. Clin. Invest. 92*:62-74 (1993)).

The second VH group, with two representatives, was 91% homologous to VH3 germline gene DP54 (Tomlinson, I.M., *et al.*, *J. Mol. Biol. 227*:776-798 (1992) (Table 1, Figs. 6(A) and (B)). The DP10-related and DP54-related VH genes are combined with JH5 and JH4 genes, respectively (Table 1) and with different D region genes. The major group of DP10-related H chains used the D region germline gene DHQ52 (Schroeder, H.W., Jr., *et al.*, *Science 238*:791-793 (1987)) (Fig. 5(C)), which is preferentially used in development of the human immune system. The D region used by the DP54-related H chains bore no resemblance to known D germline genes (Fig. 6). Partial nucleotide sequencing of the constant regions confirmed that both groups of H chains were of the IgG1 subclass.

It must be emphasized that, despite the similarity between the randomly selected orbital tissue immunoglobulin genes and TPO autoantibody genes from thyroid tissue, none of the VH and VK genes expressed in the two tissues are identical. Moreover, the H chain D regions used in the genes from thyroid tissue and orbital tissue are different, indicating different rearrangements.

A controversial issue is whether cell-mediated or humoral immunity is primarily responsible for the orbital tissue changes in Graves' ophthalmopathy (Wall, J.R., *et al.*, *Clin Immunol. Immunopathol. 68*:1-8 (1993) and Weetman,

A. P., *Autoimmunity 12*:215-222 (1992)). Autoantibodies against an orbital tissue antigen could arise as a secondary event following cell-mediated damage. On the other hand, the genesis of such autoantibodies could be a primary event. Because the IgG (and not IgM) H chain genes were investigated, the antibodies encoded by these genes resemble the IgG class antibodies characteristic of autoimmune thyroid disease (McLachlan, S.M., *et al., Endocr. Rev. 13*:192-206 (1992)), which reflect chronic stimulation of the immune system. However, regardless of the relative importance of cell-mediated or humoral immunity, B cells and T cells may both recognize the same antigen, although their epitopes may be different. Therefore, information on orbital tissue autoantibodies and their genes enable the identification of the respective autoantigen(s).

The restricted H and L chain gene usage that was observed in Graves' orbital tissue is similar to the H and L chain restriction reported for synovial tissue-infiltrating B cells from a patient with rheumatiid arthritis (Lee, S.K., *et al., Arth. Rheum. 35*:905- 913 (1992) and Bridges, JR., *et al., Arth Rheum.*, In Press (1993)). It is also remarkable that the genes encoding the dominant H and/or L chains from orbital tissue are closely related to genes which encode some autoantibodies to thyroid peroxidase and a striated muscle autoantibody. These data suggest that particular germline genes may be associated with human autoimmunity in general, or with particular manifestations of autoimmune disease, such as the tissue changes in Graves' ophthalmopathy.

Immunoglobulins and T cell receptors have similar molecular structures. This investigation of immunoglobulin variable region genes in Graves' orbital tissue is analogous to the analysis of T cell receptor variable gene usage in the thyroid of patients with autoimmune thyroid disease.

The possibility of restricted T cell receptor gene usage within the thyroid has generated much interest (Martin, A., *et al., Thyroid* 2:247-261 (1992)). The orbital tissue B cell immunoglobulin genes in the present report enables the identification of the antigens recognized by these T cell receptors.

This study provides the first information regarding the immunoglobulin genes expressed by plasma cells in the orbital tissue of Graves' ophthalmopathy. These data enable the identification of the orbital antigen(s) which may be the target of a humoral or a cell-mediated immune response.

TABLE 1

Group	L Chain (kappa)	Family	VK Germline Gene (putative)	JK
I	OF7K.3,5,9,10,11, 12,15,16,17,18,19, 20,23,25	VK 1	KL012	2*
II	OF7K.7	VK 2	VK005	1

* Except for OF7K.11 and 19 which used JK 1.

Group	H Chain	Family	VH Germline Gene (putative)	JH
I	OF7H1.2,4,5,6,7, 8,10,12,15,16,18, 20,24	VH 1	DP10	5
II	OF7H1.19,25	VH 3	DP54	4

Groups I and II were associated with different D genes (Figs. 3 and 4)

All of the publications cited herein are hereby incorporated by reference into the parent disclosure.

It will be appreciated by those skilled in the art that various modifications can be made without departing from the essential nature thereof. It is intended to encompass all such modifications within the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: NICHOLS INSTITUTE DIAGNOSTICS

(ii) TITLE OF INVENTION: GRAVES' OPHTHALMOPATHY ASSOCIATED ANTIBODIES, GRAVES' OPHTHALMOPATHY ORBITAL ANTIGEN, AND METHODS OF USE THEREOF

(iii) NUMBER OF SEQUENCES: 35

(iv) CORRESPONDENCE ADDRESS:
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(E) COUNTRY: U.S.A.
(F) ZIP: 20004

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA
(A) APPLICATION NUMBER: TBA
(B) FILING DATE: 22-SEP-1994

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(A) APPLICATION NUMBER: US 08/124,469
(B) FILING DATE: 22-SEP-1993

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 32,073
(C) REFERENCE/DOCKET NUMBER: 102105.301PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (202)942-8400
(B) TELEFAX: (202)942-8484

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 285 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GACATCGTGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CAGAGTCACC 60

ATCACTTGCC GGGCAAGTCA GAGCATTAGC AGCTATTTAA ATTGGTATCA GCAGAAACCA 120

GGGAAAGCCC CTAAGCTCCT GATCTATGCT GCATCCAGTT TGCAAAGTGG GGTCCCATCA 180

AGGTTCAGTG GCAGTGGATC TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT 240

GAAGATTTTG CAACTTACTA CTGTCAACAG AGTTACAGTA CCCCT 285

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 285 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAGCTCGTGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CAGAGTCACC 60

ATCACTTGCC GGGCAAGTCA GACCATTAGC ACCTATTTAA ATTGGTATCA GCAGAAACCA 120

GGGGAAGCGC CTAAGCTCCT GATCTATGTT GTATCCAGTT TGCAAAGTGG GGTCCCATCA 180

AGATTCAGTG GCAATGGATC TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACGT 240

GAAGATTTTG CAACTTACTA CTGTCAACAG AGTTACAGTG CCCCA 285

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 285 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAGCTCGTGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CAGAGTCACC 60

ATCACTTGCC GGGCAAGTCA GACCATTAGC ACCTATTTAA ATTGGTATCA GCAGAAACCA 120

GGGGAAGCGC CTAAGCTCCT GATCTATGTT GTATCCAGTT TGCAAAGTGG GGTCCCATCA 180

AGATTCAGTG GCAATGGATC TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACGT 240

GAAGATTTTG CAACTTACTA CTGTCAACAG AGTTACAATG CCCCG 285

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 285 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GAGCTCGTGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CAGAGTCACC 60

ATCACTTGCC GGGCAAGTCA GACCATTAGC ACCTATTTAA ATTGGTATCA GCAGAAACCA 120

GGGGAAGCGC CTAAGCTCCT GATCTATGTT GTATCCAGTT TGCAAAGTGG GGTCCCATCA 180

AGATTCAGTG GCAATGGATC TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACGT 240

GAAGATTTTG CAACTTACTA CTGTCAACAG AGTTATAGTG CCCCT 285

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 285 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAGCTCGTGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CAGAGTCACC 60

ATCACTTGCC GGGCAAGTCA GACCATTAGC ACCTATTTAA ATTGGTATCA GCAGAAACCA 120

GGGGAAGCGC CTAAGCTCCT GATCTATGTT GTATCCAGTT TGCAAAGTGG GGTCCCATCA 180

AGATTCAGTG GCAATGGATC TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACGT 240

GAAGATTTTG CAACTTACTA CTGTCAACAG AGTTACAGTG CCCCG 285

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 285 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAGCTCGTGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CAGAGTCACC 60

ATCACTTGCC GGGCAAGTCA GACCATTAGC ACCTATTTAA ATTGGTATCA GCAGAAACCA 120

GGGGAAGCGC CTAAGCTCCT GATCTATGTT GTATCCAGTT TGCAAAGTGG GGTCCCATCA 180

AGGTTCGGTG GCGGTGGATT TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACGT 240

GAAGATTTTG CAACTTACTA CTGTCAACAG AGTTACAGTG CCCCT 285

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 285 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAGCTCGTGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CAGAGTCACC 60

ATCACTTGCC GGGCAAGTCA GAGGATTAGC ACCTATTTAA ATTGGTATCA GCAGAAACCA 120

GGGAAAGCGC CTAAGCTCCT GATCTATGTT GTATCCAGTT TGCAAAGTGG GGTCCCATCA 180

AGATTCAGTG GCAATGGATC TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACGT 240

GAAGATTTTG CAACTTACTA CTGTCAACAG AGTTACAGTG CCCCG 285

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 285 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CAGAGTCACC 60

ATCACTTGCC GGGCAAGTCA GAGCATTAGC AGCTATTTAA ATTGGTATCA GCAGAAACCA 120

GGGAAAGCCC CTAAGCTCCT GATCTATGCT GCATCCAGTT TGCAAAGTGG GGTCCCATCA 180

AGGTTCAGTG GCAGTGGATC TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT 240

GAAGATTTTG CAACTTACTA CTGTCAACAG AGTTACAGTA CCCCT 285

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30

Ile Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Gln Gln Gln Ser Tyr Ser Thr Pro
85 90 95

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Thr Ile Ser Thr Tyr
20 25 30

Ile Asn Trp Tyr Gln Gln Lys Pro Gly Glu Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Val Val Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Asn Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Arg
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Gln Gln Gln Ser Tyr Ser Ala Pro
85 90 95

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Thr Tyr
20 25 30

Ile Asn Trp Tyr Gln Gln Lys Pro Gly Glu Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Val Val Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Asn Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Arg
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Gln Gln Gln Ser Tyr Asn Ala Pro
85 90 95

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Thr Ile Ser Thr Tyr
20 25 30

Ile Asn Trp Tyr Gln Gln Lys Pro Gly Glu Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Val Val Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Asn Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Arg
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Gln Gln Gln Ser Tyr Ser Ala Pro
85 90 95

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Thr Ile Ser Thr Tyr
20 25 30

Ile Asn Trp Tyr Gln Gln Lys Pro Gly Glu Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Val Val Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Asn Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Arg
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Gln Gln Gln Ser Tyr Ser Ala Pro
85 90 95

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Thr Ile Ser Thr Tyr
20 25 30

Ile Asn Trp Tyr Gln Gln Lys Pro Gly Glu Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Val Val Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Gly Gly
50 55 60

Gly Gly Phe Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Arg
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Gln Gln Gln Ser Tyr Ser Ala Pro
85 90 95

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Arg Ile Ser Thr Tyr
20 25 30

Ile Asn Trp Tyr Gln Gln Lys Pro Gly Glu Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Val Val Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Asn Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Arg
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Gln Gln Gln Ser Tyr Ser Ala Pro
85 90 95

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30

Ile Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Gln Gln Gln Ser Tyr Ser Thr Pro
85 90 95

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 300 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GATATTGTGA TGACTCAGTC TCCACTCTCC CTGCCCGTCA CCCCTGGAGA GCCGGCCTCC 60

ATCTCCTGCA GGTCTAGTCA GAGCCTCCTG CATAGTAATG GATACAACTA TTTGGATTGG 120

TACCTGCAGA AGCCAGGGCA GTCTCCACAG CTCCTGATCT ATTTGGGTTC TAATCGGGCC 180

TCCGGGGTCC CTGACAGGTT CAGTGGCAGT GGATCAGGCA CAGATTTTAC ACTGAAAATC 240

AGCAGAGTGG AGGCTGAGGA TGTTGGGGTT TATTACTGCA TGCAAGCTCT ACAAACTCCT 300

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 300 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAGCTCGTGA TGACCCAGTC TCCACTCTCC CTGCCCGTCA CCCCTGGAGA GCCGGCCTCC 60

ATCTCCTGCA GGTCTAGTCA GAGCCTCCTA AATATTAATG GATTCTCCTT TTTGGATTGG 120

TTCGTGCAGA AGCCAGGACA GTCTCCACAA CTCCTGATCT ATATGGGTTC TAATCGGGCC 180

TCCGGGGTCT CTGACAGGTT CAGTGGCATT GGATCAGGGG GAAATTTTAC ACTGAAAATC 240
AGCAGAGTGG AGGCTGGGGA TGTTGGCGTT TACTACTGCA TGCAATCTCT ACAAATACCT 300

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Phe
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95

Leu Gln Thr Pro
100

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Glu Leu Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Asn Ile
20 25 30

Asn Gly Phe Ser Phe Leu Asp Trp Phe Val Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Met Gly Ser Asn Arg Ala Ser Gly Val Ser
50 55 60

Asp Arg Phe Ser Gly Ile Gly Ser Gly Gly Asn Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Gly Asp Val Gly Val Tyr Tyr Cys Met Gln Ser
85 90 95

Leu Gln Ile Pro
100

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 294 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CAGGTGCAGC TGGTGCAGTC TGGGGCTGAG GTGAAGAAGC CTGGGTCCTC GGTGAAGGTC 60

TCCTGCAAGG CTTCTGGAGG CACCTTCAGC AGCTATGCTA TCAGCTGGGT GCGACAGGCC 120

CCTGGACAAG GGCTTGAGTG GATGGGAGGG ATCATCCCTA TCTTTGGTAC AGCAAACTAC 180

GCACAGAAGT TCCAGGGCAG AGTCACGATT ACCGCGGACG AATCCACGAGCACAGCCTAC 240

ATGGAGCTGA GCAGCCTGAG ATCTGAGGAC ACGGCCGTGT ATTACTGTGC GAGA 294

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 294 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CAGGTCCAGC TGGTGCAGTC TGGGGCTGAG GTGAAGAAGC CTGGGTCCTC GGTGAAGGTC 60

TCCTGCAAGG CTTCTGGAGG CACCTTCAGC AGCTATGCTA TCAGCTGGGT GCGACAGGCC 120

CCTGGACAAG GGCTTGAGTG GATGGGAAGG ATCATCCCTA TCCTTGGTAT AGCAAACTAC 180

GCACAGAAGT TCCAGGGCAG AGTCACGATT ACCGCGGACA AATCCACGAG CACAGCCTAC 240

ATGGAGCTGA GCAGCCTGAG ATCTGAGGAC ACGGCCGTGT ATTACTGTGC GAGA 294

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 294 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CAGGTGAAAC TGCTCGAGTC GGGGGCTGAG GTGAAGAAGC CTGGGTCCTC GGTGAAGGTC 60

TCCTGCAAGG CTTCTGGAGG CACCTTCAAT AACTATGCTA TCTCCTGGGT GCCACTGGCC 120

CCTGGACAAG GACTTGAGTG GATGGGAGGG ATCATCCCTA TCTCTGGTAA AGCAAACTAC 180

GTACAGAAGT TCCAGTGTAG AGTCACGATT ACCGTGGATA AATCCACGAG GACAGCCTAC 240

ATGGGACTGA GCAGGCTGAG ATCTGAGGAG ACGGCCGTCT ATTACTGTGC GAGA 294

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gln Val Lys Leu Leu Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Asn Asn Tyr
20 25 30

Ala Ile Ser Trp Val Pro Leu Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Ser Gly Lys Ala Asn Tyr Val Gln Lys Phe
50 55 60

Gln Cys Arg Val Thr Ile Thr Val Asp Lys Ser Thr Arg Thr Ala Tyr
65 70 75 80

Met Gly Leu Ser Arg Leu Arg Ser Glu Glu Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTAACTGGGG A 11

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GAACTAACTG GGACGCCAC C 21

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Glu Leu Thr Gly Asp Ala Thr
1 5

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 294 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GAGGTGCAGC TGGTGGAGTC TGGGGGAGGC TTGGTCCAGC CTGGGGGGTC CCTGAGACTC 60

TCCTGTGCAG CCTCTGGATT CACCTTTAGT AGCTATTGGA TGAGCTGGGT CCGCCAGGCT 120

CCAGGGAAGG GGCTGGAGTG GGTGGCCAAC ATAAAGCAAG ATGGAAGTGA GAAATACTAT 180

GTGGACTCTG TGAAGGGCCG ATTCACCATC TCCAGAGACA ACGCCAAGAA CTCACTGTAT 240

CTGCAAATGA ACAGCCTGAG AGCCGAGGAC ACGGCTGTGT ATTACTGTGC GAGA 294

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 294 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CAGGTGAAAC TGCTCGAGTC TGGGGGAGGC TTGGTCAAGC CTGGGGGGTC CCTGAAACTC 60

TCCTGTACAA CCTCTGGATT CACCTTTAAA AACTATTGCA TGAGCTGGGT CCGCCAGGCT 120

CCAGGGAAGG GGCTGGAGTG GGTGGCCAAG ATAAAGCAGG ATGGAGATGA GAAGTCTTAT 180

GTGGGCTCTG TGAAGGGGCG ATTCACCATC TCCAGAGACG ACGCCGAGAA CTCACTGTAT 240

CTGCAAATGA ACAGCCTGAG AGCCGAGGAT ACGGCTGTTT ATTACTGTGC GAGG 294

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Gln Val Lys Leu Leu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Thr Thr Ser Gly Phe Thr Phe Lys Asn Tyr
20 25 30

Cys Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Lys Ile Lys Gln Asp Gly Asp Glu Lys Ser Tyr Val Gly Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Glu Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AGAGATGGGT ACAATTTAGT CGGGGGGGGG TTTCAC 36

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Arg Asp Gly Tyr Asn Leu Val Gly Gly Gly Phe His
1 5 10

WHAT IS CLAIMED IS:

1. A Graves' ophthalmopathy associated monoclonal antibody.

2. A Graves' ophthalmopathy associated monoclonal antibody produced by the process comprising the steps of:

cloning immunoglobulin genes by PCR, said immunoglobulin genes being obtained from an orbital tissue sample from one or more patients having Graves' ophthalmopathy, said tissue sample containing orbital tissue infiltrating B cells; and

expressing the resultant cloned genes in a suitable host cell to produce Graves' ophthalmopathy associated monoclonal antibody.

3. A method of detecting Graves' ophthalmopathy orbital tissue antigen in a sample, comprising contacting an orbital tissue sample with the antibody of any one of claims 1 or 2, wherein said antibody is detectably labeled, so as to form a complex between said orbital tissue antigen present in said sample and said detectably labeled antibody, and detecting the complexed or uncomplexed detectably labeled antibody.

4. An isolated substantially pure Graves' ophthalmopathy associated immunoglobulin protein, comprising one or more immunoglobulin proteins selected from the group consisting of the immunoglobulin proteins OF7K.3, OF7K.16, OF7K.11, OF7K.9, OF7K.19 and OF7K.17 having the amino acid sequence as shown in Figure 3(B); OF7K.7 having the amino acid sequence as shown in Figure 4(B); OF7H1.2 having the amino acid sequence as shown in Figure 5(B); and OF7H1.19 having the amino acid sequence as shown in Figure 6(B).

5. The isolated substantially pure Graves' ophthalmopathy associated immunoglobulin protein of claim 4, wherein the genetic sequence encoding said protein comprises recombinant DNA.

6. An isolated nucleotide sequence coding for the Graves' ophthalmopathy associated immunoglobulin protein of claim 4.

7. The isolated nucleotide sequence of claim 6, wherein said sequence comprises genomic DNA, cDNA or RNA.

8. A recombinant DNA molecule which contains a genetic sequence that codes for the Graves' ophthalmopathy associated immunoglobulin protein of claim 4.

9. The recombinant DNA molecule of claim 8, wherein said molecule is a vector.

10. A vector comprising a nucleotide sequence that codes for Graves' ophthalmopathy associated immunoglobulin protein.

11. A host cell transformed with the vector of claim 10.

12. The host cell of claim 11, wherein said host cell is a mammalian cell.

13. A method of producing isolated substantially pure Graves' ophthalmopathy associated immunoglobulin polypeptide comprising:

(a) constructing the vector of claim 10;

(b) transforming a suitable host cell with said vector of part (a);

(c) culturing said host cell under conditions which allow the expression of said Graves' ophthalmopathy associated immunoglobulin polypeptide by said host cell; and

(d) isolating said Graves' ophthalmopathy associated immunoglobulin polypeptide expressed by said host cell in part (c); whereby an isolated substantially pure Graves' ophthalmopathy associated immunoglobulin polypeptide is produced.

14. The method according to claim 13, wherein said host cell is a mammalian cell.

15. An antibody raised against the Graves' ophthalmopathy associated immunoglobulin protein of claim 4.

16. The antibody of claim 15, wherein said antibody is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, an anti-idiotypic antibody, and an anti-anti-idiotypic antibody.

17. A pharmaceutical composition comprising the antibody of claim 16 and a carrier.

18. A method of treating Graves' ophthalmopathy comprising administering to a patient having Graves' ophthalmopathy a therapeutically effective amount of the pharmaceutical composition of claim 17.

19. The antibody of claim 15, wherein said antibody is detectably labeled.

20. An immunogenic peptide fragment comprising a portion of the Graves' ophthalmopathy associated immunoglobulin protein of claim 4, said fragment being capable of inducing the production of active antibodies specific to said Graves' ophthalmopathy associated immunoglobulin protein.

21. The immunogenic peptide fragment of claim 20 which is linked to an immunoactivating carrier.

22. A method for detecting the presence of Graves' ophthalmopathy associated immunoglobulin protein in orbital tissue comprising contacting said tissue with an amount of the detectably labeled antibody of claim 19 for a time sufficient to allow said detectably labeled antibody to form a complex with said Graves' ophthalmopathy associated immunoglobulin protein; separating complexed detectably labeled antibody from uncomplexed detectably labeled antibody, and detecting complexed detectably labeled antibody, whereby the presence of said Graves' ophthalmopathy associated immunoglobulin protein in said tissue is detected.

23. The method according to claim 22, wherein said detection is carried out by imaging *in vivo*.

24. A detectably labeled nucleotide probe, comprising a first sequence which is substantially complementary to a second sequence that specifically codes for the Graves' ophthalmopathy associated immunoglobulin protein of claim 4.

25. An isolated nucleotide sequence encoding an autoimmune associated immunoglobulin fragment, said sequence is selected from the group consisting of: the nucleotide sequence of OF7K.3, OF7K.16, OF7K.11, OF7K.9, OF7K.19, and OF7K.19 as shown in Figure 3(A); the nucleotide sequence of

OF7K.7, as shown in Figure 4(A); the nucleotide sequence of OF7H1.2, as shown in Figure 5(A); and the nucleotide sequence of OF7H1.19, as shown in Figure 6(A).

26. An isolated substantially pure autoimmune associated immunoglobulin fragment selected from the group consisting of: the nucleotide sequence of OF7K.3, OF7K.16, OF7K.11, OF7K.9, OF7K.19, and OF7K.19 as shown in Figure 3(A); the nucleotide sequence of OF7K.7, as shown in Figure 4(A); the nucleotide sequence of OF7H1.2, as shown in Figure 5(A); and the nucleotide sequence of OF7H1.19, as shown in Figure 6(A).

FIG.1A

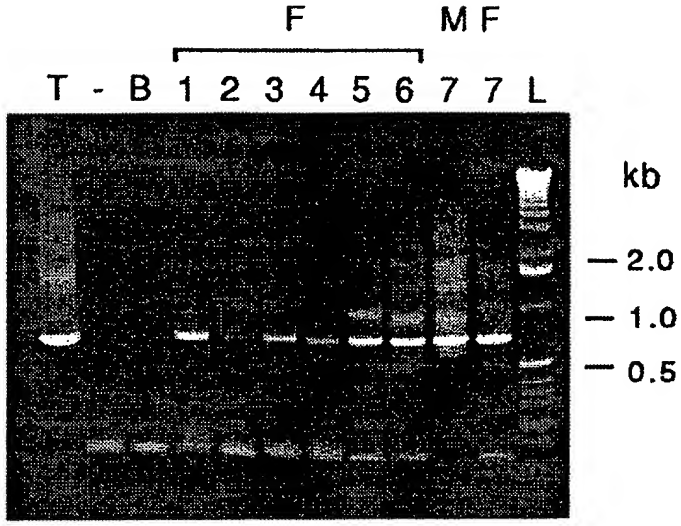


FIG.1B

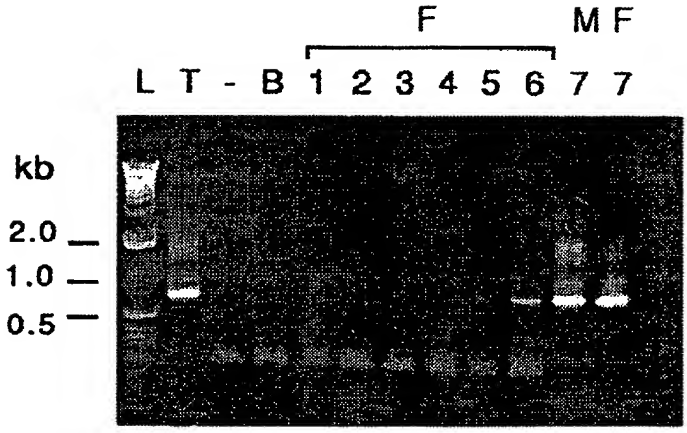


FIG.1C

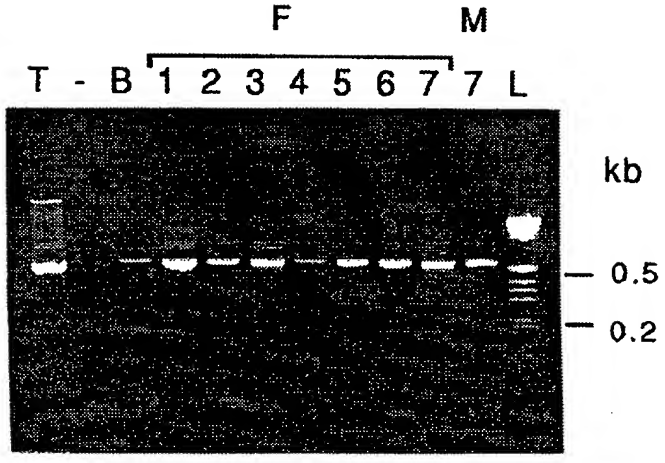
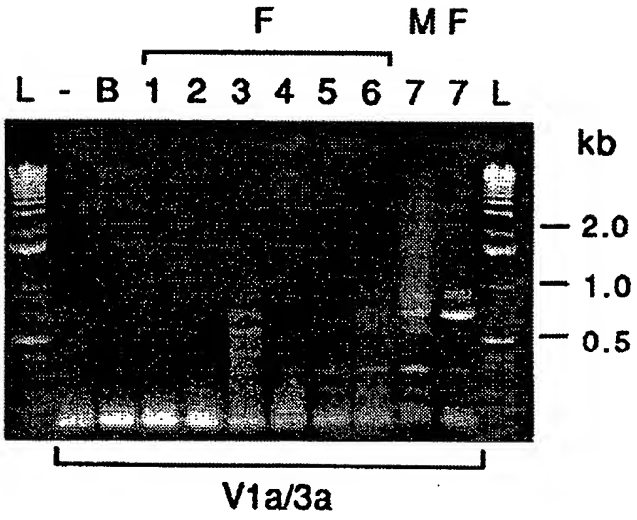


FIG.2A



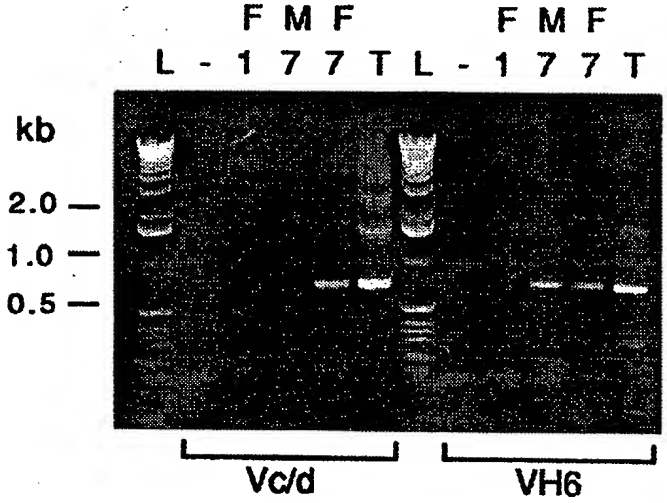


FIG.2B

```
HSIGKL012  GACATCGTGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA  50
OF7K.3     ..GC..... .......... .......... .......... ..........  50
OF7K.16    ..GC..... .......... .......... .......... ..........  50
OF7K.11    ..GC..... .......... .......... .......... ..........  50
OF7K.9     ..GC..... .......... .......... .......... ..........  50
OF7K.19    ..GC..... .......... .......... .......... ..........  50
OF7K.17    ..GC..... .......... .......... .......... ..........  50
S43434     ..CA..CA.. .......... .......... .......... ..........  50

                                          CDR1
HSIGKL012  CAGAGTCACC ATCACTTGCC GGGCAAGTCA GAGCATTAGC AGCTATTTAA 100
OF7K.3     .......... .......... .......... ..CC...... .C........ 100
OF7K.16    .......... .......... .......... ..CC...... .C........ 100
OF7K.11    .......... .......... .......... ..CC...... .C........ 100
OF7K.9     .......... .......... .......... ..CC...... .C........ 100
OF7K.19    .......... .......... .......... ..CC...... .C........ 100
OF7K.17    .......... .......... .......... ..GG...... .C........ 100
S43434     .......... .......... .......... ...C...... .G........ 100

HSIGKL012  ATTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGCT 150
OF7K.3     ..........  ..........  ...G....G.  ..........  .......T. 150
OF7K.16    ..........  ..........  ...G....G.  ..........  .......T. 150
OF7K.11    ..........  ..........  ...G....G.  ..........  .......T. 150
OF7K.9     ..........  ..........  ...G....G.  ..........  .......T. 150
OF7K.19    ..........  ..........  ...G....G.  ..........  .......T. 150
OF7K.17    ..........  ..........  ...A....G.  ..........  .......T. 150
S43434     ..........  ..........  ........C.  ..........  .......C. 150

                CDR2
HSIGKL012  GCATCCAGTT TGCAAAGTGG GGTCCCATCA AGGTTCAGTG GCAGTGGATC 200
OF7K.3     .T........ .......... .......... ..A...A... ..AA.....C 200
OF7K.16    .T........ .......... .......... ..A...A... ..AA.....C 200
OF7K.11    .T........ .......... .......... ..A...A... ..AA.....C 200
OF7K.9     .T........ .......... .......... ..A...A... ..AA.....C 200
OF7K.19    .T........ .......... .......... ..G...G... ..GG.....T 200
OF7K.17    .T........ .......... .......... ..A...A... ..AA.....C 200
S43434     .C........ .......... .......... .......... ...G...... 200
```

FIG. 3

```
HSIGKL012  TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG  250
OF7K.3     .......... .......... .......... ........G. ..........  250
OF7K.16    .......... .......... .......... ........G. ..........  250
OF7K.11    .......... .......... .......... ........G. ..........  250
OF7K.9     .......... .......... .......... ........G. ..........  250
OF7K.19    .......... .......... .......... ........G. ..........  250
OF7K.17    .......... .......... .......... ........G. ..........  250
S43434     .......... .......... .......... ........C. ..........  250

                                    CDR3
HSIGKL012  CAACTTACTA CTGTCAACAG AGTTACAGTA CCCCT                   285
OF7K.3     .......... ..........C.G.G ....A                         285
OF7K.16    .......... ..........C.A.G ....G                         285
OF7K.11    .......... ..........T.G.G ....T                         285
OF7K.9     .......... ..........C.G.G ....G                         285
OF7K.19    .......... ..........C.G.G ....T                         285
OF7K.17    .......... ..........C.G.G ....G                         285
S43434     .......... ..........A .....                            285
```

FIG.3A

```
                                    CDR1
HSIGKL012  DIVMTQSPSS LSASVGDRVT ITCRASQSIS SYLNWYQQKP GKAPKLLIYA  50
OF7K.3     EL........ .......... ......T.. T..... .E......V.      50
OF7K.16    EL........ .......... ......T.. T..... .E......V.      50
OF7K.11    EL........ .......... ......T.. T..... .E......V.      50
OF7K.9     EL........ .......... ......T.. T..... .E......V.      50
OF7K.19    EL........ .......... ......T.. T..... .E......V.      50
OF7K.17    EL........ .......... ......R.. T..... .E......V.      50
S43434     DIQ....... .......... ..... S.. S..... ........A.      50

           CDR2                                          CDR3
HSIGKL012  ASSLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYSTP       95
OF7K.3     V.....N..... .........R ..........  ...A.              95
OF7K.16    V.....N..... .........R ..........  ..NA.              95
OF7K.11    V.....N..... .........R ..........  ...A.              95
OF7K.9     V.....N..... .........R ..........  ...A.              95
OF7K.19    V..G.G.F.... .........R ..........  ...A.              95
OF7K.17    V.....N..... .........R ..........  ...A.              95
S43434     A.....S..... .........P ..........  ...T.              95
```

FIG.3B

```
                                                                  CDR1
HSIGVK005  GATATTGTGA TGACTCAGTC TCCACTCTCC CTGCCCGTCA CCCCTGGAGA  50
OF7K.7     ..GC.C.... ....C..... .......... .......... ..........  50

HSIGVK005  GCCGGCCTCC ATCTCCTGCA GGTCTAGTCA GAGCCTCCTG CATAGTAATG 100
OF7K.7     .......... .......... .......... .........A A...T.....  100

HSIGVK005  GATACAACTA TTTGGATTGG TACCTGCAGA AGCCAGGGCA GTCTCCACAG 150
OF7K.7     ...T.TC..T .......... .T.G...... .......A.. .........A  150

                                  CDR2
HSIGVK005  CTCCTGATCT ATTTGGGTTC TAATCGGGCC TCCGGGGTCC CTGACAGGTT 200
OF7K.7     .......... .A........ .......... .........T ..........  200

HSIGVK005  CAGTGGCAGT GGATCAGGCA CAGATTTTAC ACTGAAAATC AGCAGAGTGG 250
OF7K.7     ........T. ........GG G.A....... .......... ..........  250

                                                        CDR3
HSIGVK005  AGGCTGAGGA TGTTGGGGTT TATTACTGCA TGCAAGCTCT ACAAACTCCT 300
OF7K.7     ......G... ......C... ..C..... ..T..... .....TA...  300
```

FIG.4A

```
                                  CDR1
HSIGVK005  DIVMTQSPLS LPVTPGEPAS ISCRSSQSLL HSNGYNYLDW YLQKPGQSPQ  50
OF7K.7     EL........ .......... ..........NI..FSF... FV........  50

           CDR2                                                   CDR3
HSIGVK005  LLIYLGSNRA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCMQALQTP 100
OF7K.7     ...M..... ...S.....I ...GN..... .....G.... ....S..I.  100
```

FIG.4B

```
HSIGDP10  CAGGTGCAGC TGGTGCAGTC TGGGGCTGAG GTGAAGAAGC CTGGGTCCTC  50
HV1263    .....CC.G. ..G.GC.... T..........  ..........  ..........  50
OF7H1.2   .....GA.A. ..C.CG.... G..........  ..........  ..........  50

                                                          CDR1
HSIGDP10  GGTGAAGGTC TCCTGCAAGG CTTCTGGAGG CACCTTCAGC AGCTATGCTA  100
HV1263    ..........  ..........  ..........  ........GC .G........  100
OF7H1.2   ..........  ..........  ..........  ........AT .A........  100

HSIGDP10  TCAGCTGGGT GCGACAGGCC CCTGGACAAG GGCTTGAGTG GATGGGAGGG  150
HV1263    ..AG.....  ..G..A....  ..........  .G........  .......A..  150
OF7H1.2   ..TC.....  ..C..T....  ..........  .A........  .......G..  150

                                CDR2
HSIGDP10  ATCATCCCTA TCTTTGGTAC AGCAAACTAC GCACAGAAGT TCCAGGGCAG  200
HV1263    ..........  ..CT.....T  ..........  .C........  .....G.C..  200
OF7H1.2   ..........  ..TC.....A  ..........  .T........  .....T.T..  200

HSIGDP10  AGTCACGATT ACCGCGGACG AATCCACGAG CACAGCCTAC ATGGAGCTGA  250
HV1263    ..........  ....C...CA  ..........  C.........  ....AG....  250
OF7H1.2   ..........  ....T...TA  ..........  G.........  ....GA....  250

HSIGDP10  GCAGCCTGAG ATCTGAGGAC ACGGCCGTGT ATTACTGTGC GAGA        294
HV1263    ....C.....  .........C  ........G.  ..........  ....    294
OF7H1.2   ....G.....  .........G  ........C.  ..........  ....    294
```

FIG.5A

```
                                                  CDR1
HSIGDP10  QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGG  50
HV1263    ..Q.VQ.... .......... .........S S.....RQ. .........R  50
OF7H1.2   ..K.LE.... .......... .........N N.....PL. .........G  50

          CDR2
HSIGDP10  IIPIFGTANY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCAR    98
HV1263    ....L.I... A....G.... ...K..S... .E..S....D ........    98
OF7H1.2   ....S.K... V....Q.... .V.K..R... .G..R....E ........    98
```

FIG.5B

```
                CDR3 (D)
DHQ52     CTAACTG GGGA                                    11
OF7H1.2   GAA..... ....CGCCAC C                           21
          E  L  T  G   D  A  T                             7
```

FIG.5C

```
HSIGDP54  GAGGTGCAGC TGGTGGAGTC TGGGGGAGGC TTGGTCCAGC CTGGGGGGTC  50
OF7H1.19  C.....A.A. ..C.C..... .......... ......A... ..........  50

                                                        CDR1
HSIGDP54  CCTGAGACTC TCCTGTGCAG CCTCTGGATT CACCTTTAGT AGCTATTGGA  100
OF7H1.19  .....A.... ......A..A .......... ........AA .A......C.  100

HSIGDP54  TGAGCTGGGT CCGCCAGGCT CCAGGGAAGG GGCTGGAGTG GGTGGCCAAC  150
OF7H1.19  ....^..... .......... .......... .......... ......^..G  150

                                CDR2
HSIGDP54  ATAAAGCAAG ATGGAAGTGA GAAATACTAT GTGGACTCTG TGAAGGGCCG  200
OF7H1.19  .........G. .....GA... ...G.CT... ....G..... .......G..  200

HSIGDP54  ATTCACCATC TCCAGAGACA ACGCCAAGAA CTCACTGTAT CTGCAAATGA  250
OF7H1.19  .......... .........G .....G.... .......... ..........  250

HSIGDP54  ACAGCCTGAG AGCCGAGGAC ACGGCTGTGT ATTACTGTGC GAGA        294
OF7H1.19  .......... .........T ........T. .......... ...G        294
```

FIG.6A

```
                                                CDR1
HSIGDP54  EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYWMSWVRQA PGKGLEWVAN  50
OF7H1.19  Q.K.L..... ..K.....K. ..TT.....K N.C..... ........ .K  50

          CDR2
HSIGDP54  IKQDGSEKYY VDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAR    98
OF7H1.19  .....D..S. .G........ ...D.E.... .......... ........   98
```

FIG.6B

```
                    CDR3 (D)
OF7H1.19  AGAGATGGGT ACAATTTAGT CGGGGGGGGG TTTCAC   36
          R  D  G  Y  N  L  V  G  G  G  F  H      12
```

FIG.6C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10756

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/388.1, 387.2 ; 424/131.1; 435/7.1, 240.1, 252.3 and 320.1; 536/23.53, 24.33 and 23.53.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline
search terms: Graves' ophthalmopathy, Of7K.11, OF7K.9, OF7K.19, OF7K.17, OF7K.3, OF7K.16, OF7K.7, OF7H1.2, OF7H1.19, antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. CLINICAL INVESTIGATION, VOLUME 90, ISSUED SEPTEMBER 1992, PORTOLANO ET AL., "RECOGNITION BY RECOMBINANT AUTOIMMUNE THYROID DISEASE-DERIVED FAB FRAGMENTS OF A DOMINANT CONFORMATIONAL EPITOPE ON HUMAN THYROID PEROXIDASE", PAGES 720-726, SEE ENTIRE DOCUMENT.	1-26
Y	EUR. J. IMMUNOL., VOLUME 21, ISSUED 1991, PARGENT ET AL., "THE HUMAN IMMUNOLGLOBULIN X LOCUS, CHARACTERIZATION OF THE DUPLICATED O REGIONS", PAGES 1821-1827, SEE ENTIRE DOCUMENT.	1-26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 DECEMBER 1994	Date of mailing of the international search report 12 JAN 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Donald E. Adams, Ph.D. Telephone N . (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10756

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ENDOCRINE REVIEWS, VOLUME 12, NUMBER 2, ISSUED 1992, MCLACHLAN ET AL. "THE MOLECULAR BIOLOGY OF THYROID PEROXIDASE: CLONING, EXPRESSION AND ROLE AS AUTOANTIGEN IN AUTOIMMUNE THYROID DISEASE", PAGES 192-206, SEE ENTIRE DOCUMENT.	1-26
Y	AUTOIMMUNITY, VOLUME 12, ISSUED 1992, WEETMAN, "THYROID-ASSOCIATED OPHTHALMOPATHY", PAGES 215-222, SEE ENTIRE DOCUMENT.	1-26
Y	CLINICAL ENDOCRINOLOGY, VOLUME 35, ISSUED 1991, KENDLER ET AL., "A 64 kDa MEMBRANE ANTIGEN IS A RECURRENT EPITOPE FOR NATURAL AUTOANTIBODIES IN PATIENTS WITH GRAVES' THYROID AND OPHTHALMIC DISEASES", PAGES 539-547, SEE ENTIRE DOCUMENT.	1-26
Y	ACTA ENDOCRINOLOGICA, VOLUME 121, NUMBER SUPPL 2, ISSUED 1989, CAMPBELL, "IMMUNOLOGY OF GRAVES' OPHTHALMOPATHY: RETROBULBAR HISTOLOGY AND HISTOCHEMISTRY", PAGES 9-16, SEE ENTIRE DOCUMENT.	1-26
Y	ARTHRITIS AND RHEUMATISM, VOLUME 32, NUMBER 1, ISSUED JANUARY 1989, CHEN ET AL., "CHARACTERIZATION OF TWO IMMUNOGLOBULIN VH GENES THAT ARE HOMOLOGOUS TO HUMAN RHEUMATOID FACTORS", PAGES 72-76, SEE ENTIRE DOCUMENT.	1-26
Y	BIOCHEM. BIOPHYS. RES. COMM., VOLUME 179, NUMBER 1, ISSUED 30 AUGUST 1991, PORTOLANO ET AL., "A HUMAN FAB FRAGMENT SPECIFIC FOR THYROID PEROXIDASE GENERATED BY CLONING THYROID LYMPHOCYTE-DERIVED IMMUNOGLOBULIN GENES IN A BACTERIOPHAGE LAMBDA LIBRARY", PAGES 372-377, SEE ENTIRE DOCUMENT.	1-26
Y	US, A, 4,376,110, (DAVID ET AL.) 08 MARCH 1983, SEE ENTIRE DOCUMENT.	3 AND 22
Y	EP, A, 0,125,023 (CABILLY ET AL.) 14 NOVEMBER 1984, SEE ENTIRE DOCUMENT.	1-26
Y	WO, A, 86/01533 (NEUBERGER ET AL.) 13 MARCH 1986, SEE ENTIRE DOCUMENT.	1-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10756

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, f the relevant passages	Relevant to claim No.
Y	EP, A, 0,184,187 (KUDO ET AL.) 11 JUNE 1986, SEE ENTIRE DOCUMENT	1-26
Y	TIBTECH VOLUME 11, ISSUED FEBRUARY 1993, HARRIS ET AL., "THERAPEUTIC ANTIBODIES-THE COMING OF	1-26
Y	AGE", PAGES 42-44, SEE ENTIRE DOCUMENT.	
	EP, A, 0,171,496 (TANIGUCHI ET AL.) 19 February 1986, SEE ENTIRE DOCUMENT.	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10756

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 35/14, 39/00; C07K 1/00, 2/00, 4/00, 14/00, 15/00, 16/00, 17/00; G01N 33/53; C12N 5/00.

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/388.1, 387.2 ; 424/131.1; 435/7.1, 240.1, 252.3 and 320.1; 536/23.53, 24.33 and 23.53.